Assessment of the function of the central macula in diabetic maculopathy

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Medicine by

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Declaration

This thesis is the result of my own work. The material contained in this thesis has not been presented either wholly or in part for any other degree or qualification.

The clinical observations and investigations were undertaken at the Clinical Eye Research Centre under the auspices of the Department of Ophthalmology, University of Liverpool and St Paul's Eye Unit, Liverpool.

Signed……..

Ankur Raj

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Abstract

Introduction: Neural degeneration of the retina has been demonstrated in diabetes mellitus (DM). Several studies have focused on retinal neural function in diabetic retinopathy (DR) but few have assessed function in diabetic maculopathy, the leading cause of visual disturbance in DM.

Aim: To correlate, cross-sectionally and longitudinally, central macular function to diabetic maculopathy.

Methods: Treatment-naïve subjects with DM were recruited to three groups: i) diabetic controls (no visible signs of DR); ii) early maculopathy (maculopathy not meeting criteria for clinically significant macular oedema (CSMO)); and iii) sight-threatening maculopathy (presence of CSMO and/or ischaemic maculopathy). A group of healthy controls was also recruited. Subjects underwent assessment of best correct visual acuity (BCVA), contrast sensitivity (CS), optical coherence tomography (OCT), microperimetry (MP), multifocal electroretinography (mfERG), oscillatory potential (OP) and systemic risk factors (HbA1c, serum cholesterol and blood pressure). Subjects with DM were invited to follow-up at 6 months and 12 months where assessments were repeated. One-way ANOVA and ANCOVA were used for cross-sectional and longitudinal analysis, respectively (SPSS, Version 22).

Results: Eighty-nine subjects with DM (diabetic controls, n=24; early maculopathy, n=24; sight-threatening maculopathy, n=41) and 29 healthy controls were recruited. Compared to both healthy and diabetic controls, subjects with sight-threatening maculopathy showed significant worsening in CS (10-15% reduction, $p<0.01$), MP central ring sensitivity (23-29% reduction, $p<0.01$), mfERG central ring amplitude (45% reduction, $p<0.01$), mfERG implicit time (7% prolongation, $p<0.01$) and OP sum amplitude (35% reduction, $p\leq0.01$), and a 20-25% increase in mean central subfield thickness(CSFT) on OCT ($p<0.01$). Subjects with early maculopathy showed a trend towards worsening in mfERG amplitude (23% reduction, $p<0.05$), CS (7% reduction, $p<0.05$) and MP sensitivity ($p=0.06$) compared to healthy controls. Function was non-significantly reduced in diabetic controls compared to healthy controls. Sixty-one subjects were invited for follow-up, with 39 and 31 subjects attending at 6 and 12 months respectively. At 12 months, there was a trend towards worsening OP sum amplitude ($p=0.03$); conversely, MP sensitivity improved ($p<0.01$). There were no significant or trend associations with other assessments, most notably best corrected distance visual acuity. There was no correlation between change in mean central subfield thickness on OCT and change in mfERG, MP and OP ($p>0.10$ for all comparisons).

Conclusions: Central macular function is reduced in diabetic maculopathy despite reasonable visual acuity. Assessment of neural function alongside clinical examination may provide the clinician with a clearer picture of central macular status and aid in clinical decision making.

Chapter 1 - An introduction to diabetes mellitus and diabetic eye disease

Diabetes mellitus refers to a group of conditions where impairment in glucose metabolism results in elevated blood glucose levels. This results in micro- and macrovascular complications affecting multiple organs, one of them being the retina. In this introductory chapter I will review the history behind diagnosing diabetes mellitus and the features of diabetic eye disease.

1.1 History of diabetes mellitus pre-17th century

The earliest recorded description of diabetes mellitus (DM) appears to come from Ancient Egypt.[1] A physician named Hesy-Ra produced the Ebers Papyrus, a treatise on therapeutics, somewhere between 300 and 1500BC. He refers to a '*sugar disease'* in which polyuria appears to be a common symptom.[1,2] The Ebers Papyrus mentions a specific treatment of polyuria:

> *'A measuring glass filled with Water from the Bird pond, Elderberry, Fibres of the asit plant, Fresh Milk, Beer-Swill, Flower of the Cucumber, and Green Dates. Urinary troubles in the adult were also corrected with rectal injections of olive oil, honey, sweet beer, sea salt, and seeds of the wonderfruit'*.[3]

Susruta, a physician from India, appeared to make the first clinical diagnosis of DM when he reported honey urine*, madhumea*, seen in stout Hindu patients in India.[1,4] However the date of this description ranges from anywhere between 1000 BC and 6th Century AD.[1] Another Indian name for a condition resembling DM was '*prameha*' meaning urinary flux.[5] Other Hindu physicians, including Charaka and Vaghbata, described these findings along with observing how ants and flies were attracted to the urine of patients with these symptoms.[3]

In ancient Greece, Hippocrates described some signs and symptoms suggestive of DM, mentioning a disease of excessive urinary flow and wasting of the body.[3] A disciple of his, Galen, described a condition with features of "*diarrhoea of the urine*" and "*the thirsty disease*", and hypothesised this condition originated from the kidney.[1,3] His compatriot, Aretaeus of Cappadocia, an eminent physician of the Pneumatic School, introduced the term 'diabetes', *δίαβnrηs*, adapted from the Greek word *δίαβαίνω*, the verb for passer through.[1,2,6] He recognised the chronicity of this condition and he provides a comprehensive description of a condition where there is

> *'...a moist and cold wasting of the flesh and limbs into urine...The patients never cease making water...The disease is chronic in nature, and is slowly engendered, though the patient does not survive long when it is completely established...'* [1,3]

Though ancient practitioners noted that insects were attracted to the urine of some individuals they never linked this finding to the other features of polydypsia and polyuria.[1,2] Avicenna, the Arabian 'Prince of Physicians', and Rhazes described the condition, its features and its treatment in their books *Canon* and *Liber Continens*, respectively. They recognised that the urine smelt and tasted sweet through the observation that wasps, flies and ants were attracted to the urine of sufferers.[2,4] Indeed 'water tasters' were used to taste the urine of individuals suspected of having diabetes. The discovery of the sweetness of urine led to the Latin word '*mellitus*' (meaning honey) being added to the term diabetes.[1,5] At a similar time, Moses Maimonides, a rabbi, physician and philosopher, hypothesised that the 'sweet waters of the Nile and the prevailing heat' caused DM.[3]

Other features established in ancient times included excessive dry skin, reduced secretions and repeated infections (boils, carbuncles) with children dying quickly and adults developing devastating complications.[1]

Initial remedies were based around dietary recommendations (bear meat, diluted wine, herbs and grains) with no obvious basis for their implementation. Venesection was performed to reduce blood flow to the kidneys and so reduce their burden of

work. Other methods employed included avoidance of sex (to suppress overabundance of urine), bareback horse riding (as recommended by Avicenna in the 10th-11th Century AD to 'employ friction and alleviate excess urination'), and the use of opiates, tepid baths and drinking wine in the later stages of the disease. In the 1300s purgatives and astringents were prescribed by physicians to relieve polyuria and strain on the kidneys.[1]

The causative organ in DM was elusive in the first half of the second millennium with the kidneys, blood, liver and the stomach deemed as the culprits. It was only in the 16th century when Paracelsus, a Basel Physician, recognised DM to be a serious general disorder, though the cause remained uncertain. He evaporated urine to leave behind a substance he thought was salt but was likely to be glucose.[2] Paracelsus led the Renaissance physicians who introduced science into the understanding of medicine. They challenged authority and undertook scientific studies to understand anatomy and bodily functions.[3] However, the management of DM underwent little progress; for example dietary suggestions included foods high in fat and carbohydrate content, reflecting the poor understanding of the condition.[1]

1.2 Diabetes mellitus between the 17th and early 19th century

The first mention in European medical literature of 'sugar in urine' was in the 17th Century.[1] A British physician, and sometime Sedleian Professor of Natural Philosophy at Oxford, Thomas Willis (1621-1715), tasted the urine of a patient and noted its sweetness in his celebrated book '*Pissing Evil*'.[1,2,4,5] He also proposed that glycaemia, the presence of glucose in blood, preceded glycosuria in DM.[3] He recommended a diet consisting of milk, bread and barley water, i.e. one that was high in carbohydrates but low in calories. Twenty years later he appears to have changed his mind and suggested a high fat, high protein and low carbohydrate diet. However there was once again little progress for another century.[1]

In the 1770s Dr Matthew Dobson of Liverpool Infirmary confirmed Willis' findings and theories by discovering the presence of sugar in the bloodstream, suggesting a systemic disease.[2] He heated 'two quarts of urine to dryness' and noted a

granulated residue that smelt like brown sugar.[3] He also noted that in patients with diabetes their kidneys appeared to filter a considerable amount of "saccharine matter".[2] He presented his findings to the Medical Society of London in 1776.[3] Twelve years later a researcher called Thomas Cawley correlated the presence of DM with the discovery of a 'shrivelled pancreas' when performing an autopsy on a patient with DM. This finding, however, went unheeded and a general surgeon, John Rollo, placed further emphasis on the stomach being the culprit in 1796. His theory was based on his observations of a diet high in meat being associated with reduced glycaemia, whilst bread, fruits and grain resulted in increased sugar production. These findings were corroborated by French Physician, Apollinaire Bouchardat, in 1870 who noted that soldiers with DM had reduced glycosuria when food was rationed during the Franco-Prussian War.[1] Other causes of DM suggested at the time included drinking cold water when one was hot, drinking excess alcohol, suffering from excessive anxiety, or as a direct result of a physical assault.

During the 19th Century the management of DM was based on the stringent attention to diet. The aim was to avoid foods containing sugars and starch and consuming meat and green vegetables. Unfortunate individuals were denied staple foods such as potatoes, carrots, peas, bread and pasta. Some were allowed bran cakes in place of bread. They were allowed non-stimulating beverages such as tea, coffee, soda and water, but were denied lemonade, sweet wines and sweet ales. It was also suggested that drinks were to be taken warm to alleviate the craving for liquids. Other treatments included the use of opium given as compound soap pills to reduce urine production, phosphoric acid, bromide of potassium and nitrate of uranium. Extract of ergot was offered, and so was a skimmed milk diet which consisted of six pints of skimmed milk per day as the only form of nourishment for six consecutive weeks with animal food allowed after that. Relief from this strict diet included a warm bath once or twice weekly or a Turkish bath.[1]

1.3 Diabetes mellitus from the mid-19th century onwards

The understanding of the pathophysiology of DM developed rapidly in the latter half of the 19th Century. Claude Bernard hypothesised the role of the liver in

glycogen storage and glucose production early in that century through his work on animal glucogenesis. He isolated 'glycogen' from the liver, thus paving the understanding of the role of the liver in DM. He considered the overproduction of glucose to be the cause of DM.[1-3]

In 1880 a French physician, Lancereaux, identified two variants of DM. He labelled them as 'maigre' (thin) and 'gras' (fat). This paved the way to linking obesity to excess sugar intake and the identification of type 2 DM.[5]

In 1869 Paul Langerhans identified the presence of islet cells in the pancreas whilst still a medical student.[1,2] However he died in 1888 before he could explain their significance. The following year two Germans, Joseph von Mering and Oscar Minkowski, identified the pancreas as the essential organ in glucose metabolism and thus to be the major organ in DM. They showed that a dog developed DM following removal of the pancreas.[2] In 1893 a French doctor, Gustave Laguesse, suggested the role of the islet cells of the pancreas in DM and named them the 'Islets of Langerhans' after their discoverer. He hypothesised that the product released by these cells is important in controlling blood glucose levels and a lack of this product leads to DM. Thus the concept of type 1 DM was realised.

Clinicians were also gaining a greater understanding of the disease. The 'great loud breathing' was identified by Kussmaul to be an indicator of severe metabolic imbalance due to uncontrolled blood sugar levels.[2] Following this knowledge, Naunyn and Magnus Levy insisted that in the absence of carbohydrates the body imperfectly metabolised fats resulting in the formation of ketones and acids that are now synonymous with the life-threatening condition of diabetic ketoacidosis.[2] Naunyn recognised that sugar-free urine was associated with greater sugar tolerance and so recommended a diet that was reduced in carbohydrates and calories.[2]

1.4 The discovery of insulin

Across the Atlantic Ocean, an American vivisectionist, Moses Barron, identified that the islet cells were damaged in patients with DM and suggested the product

secreted from these cells could be used for treatment.[1] This product was named 'insuline' after the Latin word *insula*, meaning island, by the English physiologist, Sir Edward Albert Sharpey-Schafer, in 1910.[1] After several failed attempts to extract insulin, Zuelzer isolated insulin and used it to treat humans. However he had encountered several alarming side-effects and so abandoned treatment.[2]

Eventually a group from the University of Toronto achieved success. In 1922 Frederick Banting, Charles Best and John Macleod isolated insulin from dogs and they were able to inject it into dogs with diabetes to control their hyperglycaemia.[1,2] However this insulin was not suitable for human use. They recruited a biochemist, James Collip, and together were able to extract a suitable sample of insulin from cattle. The first individual to receive this treatment was a 14 year old boy named Leonard Thompson, who lived a reasonable life until his early death from pneumonia at the age of 27. Their hard work culminated in the Nobel Prize in 1923 in Physiology and Medicine.[1]

Once isolated, the structure of insulin was studied. John Jacob Abel identified its crystalline structure in 1926, contributing to the understanding of protein chemistry.[3] In 1958 Frederick Sanger was awarded the Nobel Prize in Medicine for his work in defining the exact amino acid sequence of insulin.

1.5 The insulin revolution

Prior to insulin, the only treatment was a diet with limited carbohydrates. Mortality was high amongst these patients with few surviving to marriage or procreation.[2] Insulin offered sufferers hope in their fight against this wasting disease. A nurse describes the atmosphere at the time:

> *'...the mere illusion of new hope cajoled patient after patient into new life...it was a resurrection, a crawling stirring, as of some vague springtime'* [7]

Insulin was first produced in Britain in 1923 after several trials and risk assessments. Animal insulin was the mainstay of insulin treatment till the 1980s when synthetically manufactured human insulin was made available.[1,3] The 20th Century

revolutionised the management of DM through a greater understanding of the pathophysiology of DM, the introduction of various types of insulin, the introduction of oral hypoglycaemic agents and greater flexibility available to patients in managing their DM.[7] With more sufferers living to an older age and an increasing prevalence of DM, future treatments are being aimed towards the early identification of the disease and eventually towards its elimination.

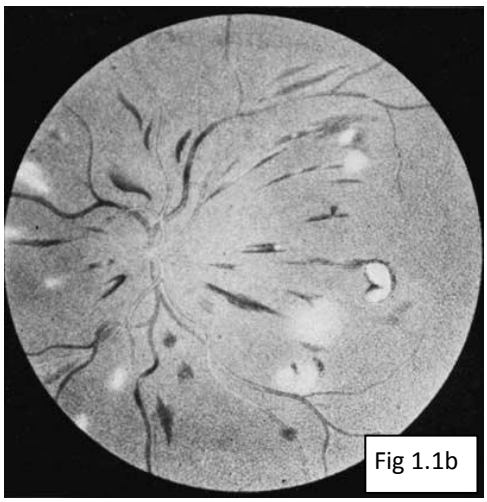
1.6 Early association between diabetes mellitus and eye disease

The first clinician to link eye disease to DM was the French ophthalmologist and Professor of Hygiene in Paris, Appolinaire Bouchardat, in 1846.[6,8] He noted that patients with diabetes complained of visual symptoms in the absence of anterior segment pathology or cataract and their symptoms improved with better control of their blood sugar levels. His observations were also described by François Tavignot. Neither clinician was able to corroborate their observations to clinical findings or histopathological specimens.[6,8]

The invention of the ophthalmoscope aided clinicians in assessing the posterior segment of the eye.[6] Early ophthalmoscopes were complicated and were difficult to use in examining the fundus. In 1855, Jaeger (1818-1884) observed abnormalities in the macula that he attributed to DM and used a meticulous approach to recording the smallest details in his clinical findings (Figure 1.1).

In one patient he identified “roundish or oval, yellowish spots and extravasations that permeated part or the whole thickness of the retina”.[8] His diagrams were used to produce a colour atlas of 21 fundus paintings, taking 20 to 40 clinical sessions per patient to detail his illustrations.[6] However Albrecht von Graefe (1828-1870) and his contemporaries disputed these findings, citing that there was no proof of a cause-effect relationship between DM and retinal complications. Jaeger’s one supporter was Louis Desmarres who published a small report in 1858.[6,8]

Figure 1.1: (a) The first fundus drawing of diabetic macular changes was published by Eduard Jaeger in the middle of the 19th century. (b) Vascular sheathing and dilatation, intraretinal haemorrhages as well as "hard" and "soft" exudates can be observed. (From: Jaeger E. Beitr zur Pathol des Auges. Wien: 1856, p. 33 Fig. 12).[8]



A decade later, in 1869, Henry Noyes (1832-1900) published an article ('*Retinitis in glycosuria'*) supporting the link between DM and abnormalities in the macula.[6,8] In 1872, Edward Nettleship (1845-1913) provided further strength to the role of DM in the development of eye disease when he presented histological proof of cystoid macular degeneration in diabetic patients in his seminal paper '*On oedema or cystic disease of the retina'*.[6] In 1877 he published further findings in collaboration with Sir Steven Mackenzie on the abnormal retinal changes in diabetes in the report '*Glycosuric retinitis*' (Figure 1.2).[8]

Around the same time Bouchardat published his book '*De la glycosurie ou diabète sucré*', in which he described the presence of fluid and lipid accumulation within the macula resulting in 'glucose-induced amblyopia' (Figure 1.3).[8] In addition a German ophthalmologist, Theodor Leber, published a series of his clinical observations which he termed 'glycosuric retinitis'.[8]

The diagnosis of proliferative diabetic retinopathy (PDR) appeared at a similar time. Wilhelm Manz published his seminal paper, '*Retinitis Proliferans'* in 1876. He

presented drawings of vitreoretinal adhesions, fibrovascular proliferation and degeneration of the optic disc (Figure 1.4).

Figure 1.2: (a) First published drawing by Sir Steven Mackenzie and Edward Nettleship of a retinal microaneurysm in a histopathological specimen from a diabetic patient (From Mackenzie and Nettleship, Roy Ophthal Lond Hosp Rep, 1877). (b) Edward Nettleship F.R.S. (1845–1913) published the first report with histopathological proof of diabetic changes in the retina in 1872. (From: The History of Moorfields Eye Hospital, London, 1929).[8]

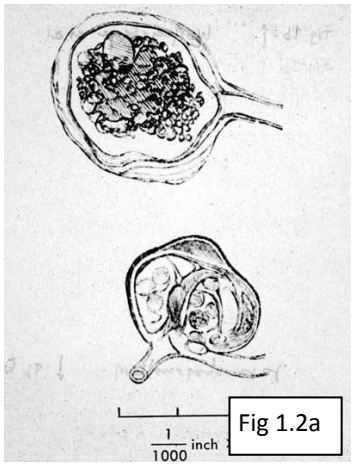


Fig 1.2a

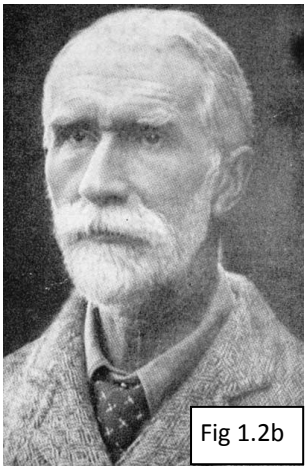


Fig 1.2b

Figure 1.3: Fundus drawing of a right eye showing advanced diabetic maculopathy. Note the presence of a large plaque of exudates (B,C) in the macula as well as several small haemorrhages (C). (From: A Bouchardat "De la glycosurie ou diabète sucré", Paris, 1875).[8]

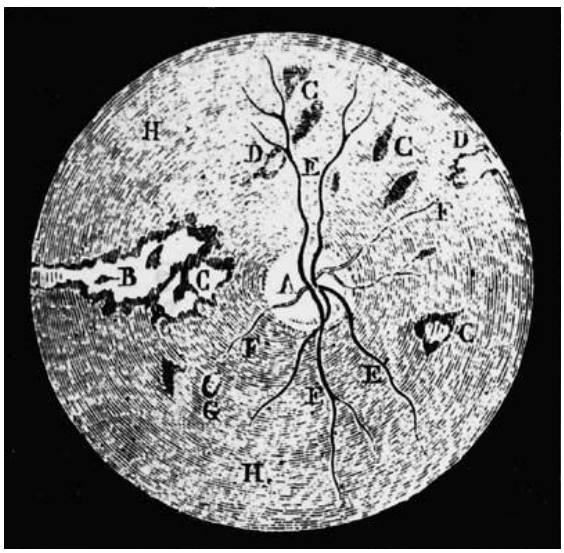
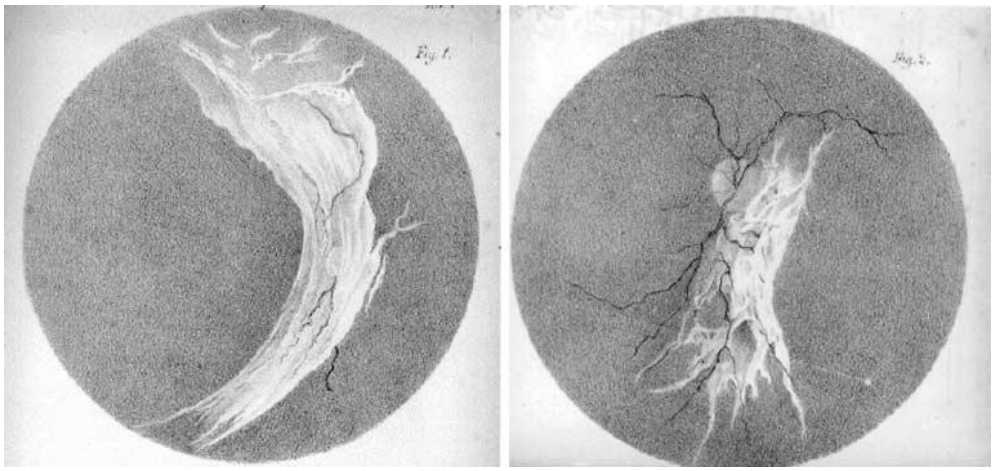


Figure 1.4: First published drawings of fibrovascular proliferations along the blood vessels in a case of proliferative diabetic retinopathy by the German ophthalmologist Wilhelm Manz. (From W. Manz, Retinitis proliferans, Graefes Arch für Ophthalmol, 1876;22, 229). [8]



The earliest classification of PDR appeared in 1890 with Julius Hirschberg dividing it into four broad groups: retinitis centralis punctuate, haemorrhagic form, retinal infarction and haemorrhagic glaucoma.[6,8]

The link between clinical findings and pathology was advanced by Arthur James Ballantyne (1876-1954) in the early part of the 20th Century (Figure 1.5).[6,8] Upon retirement he dedicated his time to understanding the microvascular changes associated with diabetes. He demonstrated abnormalities in the capillary wall, which we recognise today as loss of the inner blood retinal barrier, that result in the leakage of fluid and exudates into the various layers of the retina.[8] This paved the way in our understanding of diabetic maculopathy. I explore this further in Chapter 2.

Figure 1.5: Arthur James Ballantyne (1876–1954) chaired the ophthalmology department of the University of Glasgow. He published his most important work very late in his academic career.

1.7 Use of lasers in managing diabetic eye disease

With the identification of diabetic retinopathy and maculopathy, focus turned to the treatment of these conditions. Paradoxically, the discovery of insulin led to improved survival and increased numbers of patients developing diabetic eye complications.[8] The observation of the damaging effects of a solar eclipse on the retina prompted the German ophthalmologist, Gerhard Meyer-Schwickerath (1920-1992), to research the role of light in the treatment of retinal disorders (Figure 1.6a).[6,8] He noted that the retinal scars produced were useful in treating retinal holes and tears.

In 1949 he designed a device that was able to concentrate sunlight to a focal point on the retina, a photocoagulator. However there was limited control of delivery of treatment resulting in several excessively treated eyes and worsening vision. Eventually he was able to replace sunlight with xenon lasers developed by Zeiss Laboratories (Figure 1.6b).

Figure 1.6: (a) Gerhard Meyer-Schwickerath introduced the xenon coagulator to treat retinal diseases and thus laid the basis for the current use of laser therapy for diabetic retinopathy. (b) One of the first xenon coagulators used in the treatment of retinal disorders.


Fig 1.6a

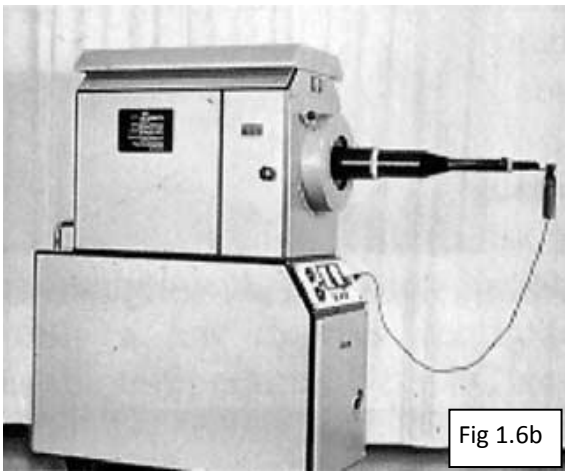


Fig 1.6b

Paul Wetzig and colleagues were the first to utilise light in a clinical setting in diabetic eye disease, using the xenon photocoagulator to destroy retinal blood vessels. However the wide beam of the xenon laser was not useful for treating small lesions and was often associated with poor outcomes. This was in part because in this period the common practice was to directly treat the proliferating vessels. Ruby lasers were then introduced into clinical practice by Christian Zweng and colleagues with some success.[6] Once again these lasers were found to be useful for retinal tears but lacked the precision in treating diabetic vascular changes.

1.7.1 Argon laser

In 1966 Zweng and Francis L'Esperance recognised that argon laser was absorbed by blood vessels, unlike ruby lasers. Photocoagulation using argon lasers showed excellent results with up to 90% of patients maintaining vision in small clinical studies.[9] Several years later the clinical effectiveness of these lasers in treating PDR was confirmed by William Beetham and Lloyd Aiello as they led a pivotal multicentre study, the Diabetic Retinopathy Study (see Chapter 3). One of the key breakthroughs was the recognition that indirect treatment to the retina produced better results compared to direct treatment of retinal vessels.

During this period ophthalmologists recognised that argon laser was useful in treating diabetic maculopathy though photocoagulation often resulted in poor outcomes due to foveal damage. As techniques were refined, several case reports and case series were published that demonstrated the beneficial effects of argon laser photocoagulation in treating diabetic macular oedema. In the early 1980's, another multicentre study performed by the Early Treatment Diabetic Retinopathy Study (ETDRS) Research Group provided clinicians with conclusive evidence and guidance on using lasers in treating diabetic macular oedema (see Chapter 3).[6,8]

1.8 Surgical treatment strategies

The surgical treatment of PDR lagged behind laser treatment. Following the observation that the progression of PDR appeared to be delayed following pituitary necrosis in a patient, empirical hypophysectomy was performed in such patients in the late 1950's.[6] Though the operation was effective in 30% of cases, it was abandoned in favour of laser therapy.[8] Robert Machemer introduced pars plana vitrectomy (PPV) for the treatment of vitreous haemorrhage secondary to PDR.[6] The role of PPV was expanded to include the delamination of fibrovascular membranes which resulted from the proliferation of retinal neovascularisation into the vitreous humour.[8] However no surgical strategies have been identified to successfully manage diabetic maculopathy.

1.9 Summary

I have briefly reviewed the history of DM and diabetic retinopathy. The rapid advances in our understanding of diabetic retinopathy and maculopathy have paved the way for developing treatment strategies that aim to minimise visual loss in individuals with this condition. In the next chapter I demonstrate the impact of diabetic maculopathy, summarise knowledge of macular anatomy and introduce the theories underpinning my research objectives.

Chapter 2 The impact of diabetes mellitus

In this chapter I describe the socioeconomic impact of diabetes mellitus and diabetic eye disease. I describe the anatomy of the retina and the pathophysiology of diabetic eye disease, in particular diabetic maculopathy. I also introduce the neurodegenerative theory of diabetic retinopathy.

2.1 The burden of disease

Diabetes mellitus (DM) is a metabolic disorder affecting approximately 220 million people worldwide.[10] It is estimated that the numbers affected will reach 365 million by 2030.[11] In the UK there are 2.4 million people with DM with prevalence likely to exceed 4 million by 2025.[12,13]

A cross-sectional study of subjects with DM, who attended the Liverpool screening programme, was performed to determine rates of diabetic eye disease in the local population.[14] Point prevalence of DM was 12.4/1000. There were 149 subjects with type 1 DM, 40 with type 2 insulin requiring DM and 268 with type 2 non-insulin requiring DM. Rates of CSMO were 2.3%, 16.2% and 5.7% respectively, with an overall prevalence of 6.4%. Furthermore, 9.2% of subjects had exudates within 1 disc diameter of the fovea or significant circinate maculopathy.

Longitudinal studies have demonstrated that nearly all patients with type 1 DM and 60% of those with type 2 DM develop some degree of diabetic retinopathy (DR) within 20 years of diagnosis.[15] The prevalence of diabetic macular oedema (DMO) was low in the first five years following diagnosis of DM (0% and 3% in Type I and Type II respectively).[16] This increased to between 20% and 30% of all patients with DM after 20 years.[15-17] A longitudinal, prospective study of 133 subjects recently diagnosed with type 2 DM demonstrated that 21% of them developed signs of diabetic maculopathy and this was associated with a decrease in visual acuity (VA).[18] Poor glycaemic control appeared to be the most important predictor.

Despite established screening programmes DR remains the leading cause of blindness amongst the working population.[17-19] Approximately 2% of patients developed blindness after 15 years of suffering from DM, with another 10% developing visual impairment.[20] Unfortunately this visual impairment is usually irreversible.[21]

Diabetic eye disease can be divided into two broad groups – diabetic retinopathy (DR) and diabetic maculopathy. Diabetic maculopathy is the leading cause of visual impairment in patient with DM.[15,18] Approximately half of all patients with diabetic maculopathy lose ≥2 Snellen lines of visual acuity (VA).[22] Chronic maculopathy results in irreversible anatomical changes to the neurosensory retina and retinal pigment epithelium resulting in permanent visual impairment.[22]

Quality of life studies have demonstrated the negative impact diabetic maculopathy has on the individual's general and mental health.[17] A population-based study in Sweden[10] estimated the costs of treating DMO without and with proliferative diabetic retinopathy (PDR) to be €216 and €433 per patient per year, respectively, highlighting the economic burden of this condition. Hence the ability to diagnose and manage diabetic maculopathy prior to visual loss will benefit both the individual and the state.

2.2 The normal macula

The macula is the region of the retina designed for central vision, colour perception and visualising fine details.[23,24] It comprises of the central 6mm of the posterior pole of the retina centred on the fovea. Histologically it is distinct from the surrounding retina by the presence of two layers of ganglion cells in the neurosensory retina.

The macula can be divided into three zones. The central zone is the fovea, measures 1500µm in diameter, and consists of the foveola (measuring 350µm in diameter). The fovea is designed for highest visual acuity. Surrounding the fovea is the parafovea which measures 2500µm in diameter. The zone from the outer edge of the parafovea to the outer edge of the macula is the perifovea.[24]

Cross-sectional imaging of the macula reveals a depression in the centre of the fovea, the foveal pit. In this unique structure the photoreceptors are tightly packed and their axons run obliquely. They connect to ganglion cells located around the foveola which themselves are arranged into a layer 6-8 cells thick. This arrangement ensures that light is not scattered by passing through other retinal layers and so maximal acuity is reached.[25,26]

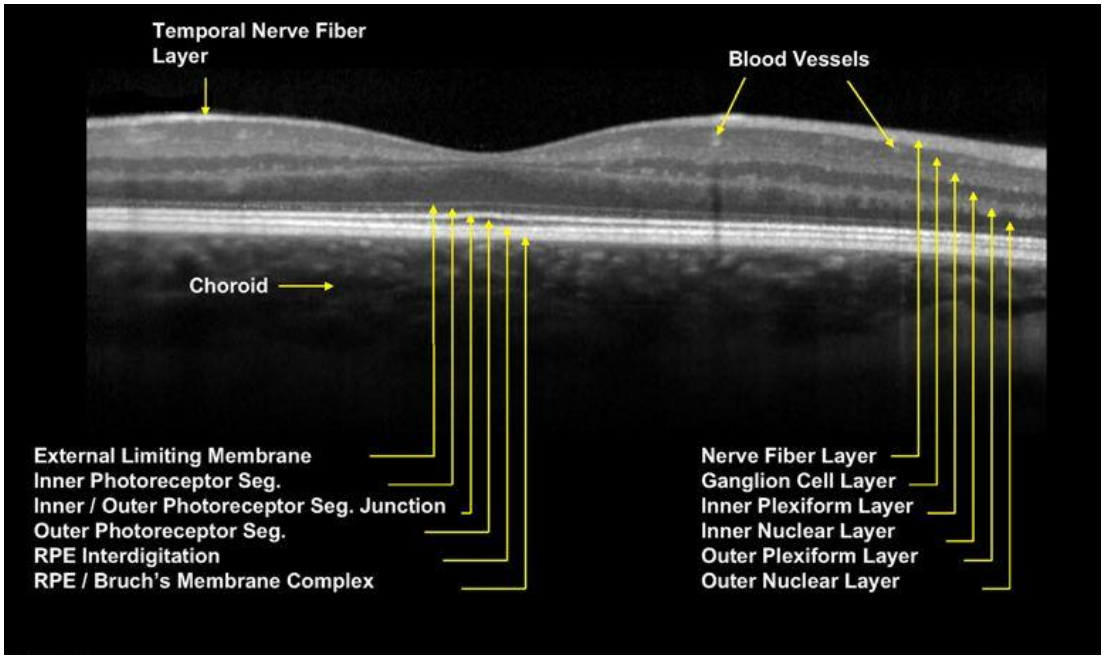
At the nasal edge of the macula lies the optic disc, a cross-sectional view of the distal end of the optic nerve. The optic disc measures on average 1800µm in diameter vertically and 1500µm horizontally, though post-mortem studies have shown the diameter of a normal optic disc to range from 900µm to 2600µm. The temporal edge of the optic disc lies approximately 3000µm nasal to the centre of the fovea.[24] A more consistent measure of anatomical relationship is the distance between the centre of the fovea and the centre of the optic disc, which measures on average 3800µm, with a range of 3000 to 4500µm.

2.3 Histology of the macula

The retina can be histologically divided into the neurosensory retina and the retinal pigment epithelial layer. The neurosensory layer is composed of a network of neural cells that are responsible for transforming light energy into 'electrical' energy (photoreceptors) and then transmitting these electrical signals to the optic nerve (bipolar, horizontal, amacrine and ganglion cells) via the nerve fibre layer (NFL).[25]

The neurosensory retina may be further subdivided into five layers reflecting the structures included within them. The outer nuclear layer (ONL) consists of the photoreceptors, i.e. the rods and cones, in particular their inner segments. The inner nuclear layer (INL) consists of horizontal cells, bipolar cells, amacrine cells and Müller cells. The ganglion cell layer (GCL) consists of ganglion cells and displaced amacrine cells. The outer plexiform layer (OPL) consists of the neural connections between photoreceptors and horizontal and bipolar cells. The inner plexiform layer (IPL) consists of neural connections between the bipolar and amacrine cells and ganglion cells.[25] Optical coherence tomography (OCT) allows for visualisation of these retinal layers (Figure 2.1).

Figure 2.1: Cross-sectional image of the macula through the centre of the fovea, as generated by optical coherence tomography, to demonstrate the different retinal layers. The photoreceptor layers are now referred to as the ellipsoid zone (Image from Heidelberg website)



Within the neurosensory layer are two capillary networks that supply the neural cells. In the human central retina, the innermost capillary plexus lies within the NFL and the outer plexus lies within the GCL. At the foveal slope there is only one capillary plexus which is located at the border of the GCL and IPL.[27] These vascular networks are essential for meeting the metabolic requirements of the retina.[25]

2.4 Physiology of the macula

The role of the retina is for vision. It is designed to convert light into electrical signal through the photoreceptors.[23] Upon stimulation by light the chromophore, retinal, undergoes changes in structure which leads to a cascade of events resulting in activation of an enzyme, phosphodiesterase. Activation of this enzyme results in reduction in levels of cyclic guanosine monophosphate (cGMP) and then the closure of sodium channels. The resulting hyperpolarisation of the photoreceptor leads to decrease in glutamate. This decrease results in depolarisation of the on-bipolar cells and hyperpolarisation of the off-bipolar cells. The electrical signal then travels

through the neural cells to the ganglion cells and the visual pathway to visual cortex of the brain.

For the neural cells to function effectively, along with meeting demanding metabolic requirements, the retinal capillary network has to maintain a "highly defined microenvironment conducive to neurotransmission, phototransduction and the complex interaction of metabolites, growth factors and vasoactive agents".[26]

Retinal homeostasis is maintained by the integrity of the inner and outer blood-retinal barriers (BRB).[28] The inner BRB is composed of the tight junctions between the endothelial cells of the retinal capillaries. This is supplemented by the pericytes that encircle capillaries.[29] The inner BRB controls the permeation of products through the retinal vasculature whilst anionic pumps located within the endothelial layer assist in the removal of excess fluid and waste product from the extracellular space.[26]

The outer BRB is formed by tight junctions between the retinal pigment epithelial (RPE) cells.[29] The RPE is involved in maintaining the normal physiological process of the retina, especially the renewal and maintenance of the photoreceptors and removal of excess fluid from the subretinal space.[29] In a study comparing retinal vasculature between patients with DM, there appeared to be no significant difference in the perifoveal capillary network and the foveal avascular zone between patients with and without diabetic macular oedema.[30] The author hypothesised that the RPE and the choroid may be implicated in the development of DMO and that choroidal ischaemia may be associated with fluid leakage. It is likely that both the inner retina and the choroid are implicated in the development of DMO.

2.5 Microvascular pathology of diabetic maculopathy

The commonest accepted theory for the development of diabetic retinopathy (DR) is the microvascular theory which attributes visual loss to changes in the retinal microvasculature, especially the capillaries.[31] These changes are also noted in the macula and results in diabetic maculopathy.

Diabetic maculopathy consists of two principal components, retinal ischaemia and retinal oedema. Retinal ischaemia results from capillary and arteriolar non-perfusion; retinal oedema is due to the breakdown of the inner or outer blood-retinal barrier resulting in a reduced ability to clear fluid transudate.[29,32] Laboratory studies have shown that there is a reduction in the quantity and quality of the tight junction proteins described above, resulting in DMO.[33] Retinal oedema may be associated with exudates, plasma lipoproteins released from damaged capillaries, and are usually located in the OPL and ONL.[32]

DM is associated with an increased inflammatory response.[34] Leukocyte release, and subsequent adherence to the endothelium, results in cell death, vascular obstruction and vascular leakage. In addition there is an increased release of vascular endothelial growth factor (VEGF) which results in increased vascular permeability. The superficial inner retinal capillary network appears to be the most susceptible vascular pathway to elevated levels of VEGF. Other vasoactive factors implicated include protein kinase C (which is associated with decreased retinal blood flow by inducing vasoconstriction), angiotensin II, vasopressin and bradykinin. Matrix metalloproteinases have been shown to influence the endothelial cell junctions and participate in endothelial and pericyte cell death.[28,34]

The oedema in diabetic macular oedema (DMO) comprises intracellular swelling of the Müller cells, the OPL or the Henle layer and the accumulation of extracellular fluid in cystoid macular oedema (CMO) mainly in the OPL and INL of the retina within the fovea.[35,36] CMO appears on fluorescein angiography (FA) as petalloid hyperfluorescence in the region of fovea in the late images. Persistent retinal oedema may result in apoptosis of Müller cells and formation of cystic cavities,[37] which involves the whole retina and may result in loss of identifiable structures, such as the fovea.[30] Several studies have demonstrated that not all patients recover vision despite adequate treatment and resolution of maculopathy and suggest this is caused by the loss of these retinal neuronal cells.

Increased blood glucose, presumed hypoxia and systemic hypertension are associated with arterial dilatation.[33] There is an increase in hydrostatic pressure

which contributes to small vessel dilatation and transudation of fluid and macromolecules, such as proteins and lipids, into the retinal interstitial spaces. These lipoprotein complexes are called exudates and are indication of either active or recent increased vascular permeability or leakage.

In addition to ischaemia and oedema, there are other features associated with diabetic maculopathy. Vitreomacular interface abnormalities (VMIA) refer to the attachment of the posterior hyaloid membrane of the vitreous to the retinal nerve fibre layer resulting in macular abnormalities. VMIA has been noted in 2.8 to 3.6% of cases of DMO[38] and the risk of developing DMO was 3.4-fold lower in those individuals in whom there was complete detachment of the posterior hyaloid surface.[39] Serous foveal detachment describes the presence of leaked fluid between the ONL and the RPE layer, has been found in up to 15% of patients with diabetes and is associated with dysfunction of the RPE.[35]

2.6 Types of diabetic maculopathy

Diabetic maculopathy may be subdivided into four types: focal exudative, diffuse, ischaemic, and mixed. Focal exudative maculopathy refers to the presence of a well circumscribed area of retinal thickening with associated exudates, usually due to leakage from microaneurysm(s) (MA) from an intraretinal vessel or dilated capillary segments at the level of the outer nuclear layer.[32,38,40,41] Diffuse maculopathy is the presence of a poorly circumscribed area of retinal thickening with possible areas of retinal tissue non-perfusion, functional dilatation of the capillaries and cystoid changes; the leakage usually results from a generalised breakdown of the inner BRB with the absence of exudates reflecting the leakage of smaller molecules.[22,32,39,41] Ischaemic maculopathy is the constriction or loss of the terminal arterioles of the perifoveal capillary network resulting in reduced oxygen and nutrient supply and thus visual loss.[22] Mixed maculopathy refers to the presence of diffuse oedema and ischaemia concurrently;[39] the term has been falling out of use in recent years.

The different types of maculopathy are determined by combining findings of clinical examination and FA. Focal exudative and diffuse maculopathy are usually described clinically though FA aids in determining involvement of the fovea. Ischaemic

maculopathy may be suspected on clinical examination through the presence of blot haemorrhages and cotton wool spots in the perifoveal region. However FA is essential for identifying any disruption to the perifoveal capillary network and FAZ.

2.7 Management of diabetic maculopathy

There are various treatment modalities available for managing diabetic maculopathy. The first line of treatment is usually laser photocoagulation, either in a targeted fashion (focal) or in a pattern of spaced burns (grid) around the FAZ, as established by the Early Treatment Diabetic Retinopathy Study (ETDRS) Group.[35] Application of laser is thought to reduce macular oedema through closure of the adjacent microaneurysms and the proliferation of the RPE cells and thus re-establishment of the outer BRB. Other possible theories are the photocoagulative debridement of unhealthy RPE cells, to be replaced by healthier cells, and the reduction of oxygen demand by the retina through the reduction in active photoreceptors.[32]

Focal laser is beneficial in reducing the rate of moderate vision loss by ~50% (from 24% to 12%) but offers little benefit in improving VA.[22,35,42] Despite treatment 12% may still lose vision.[22,35] Also, photocoagulation may be of no benefit in up to 50% of cases.[43] I describe these results in more detail in Chapter 3.

The use of peri-ocular or intra-ocular injection of steroid, such as triamcinolone, have shown benefit in reducing macular oedema and improving visual acuity. They produce anti-inflammatory effects that modify the blood-ocular barrier and inhibit vascular endothelial growth factor (VEGF) resulting in modulation of the vascular permeability. However the benefits are often short-lived, multiple treatment are often required, and an associated increased risk of adverse events including infection, glaucoma and cataract.[15,22]

Since starting my research, newer treatment strategies for diabetic maculopathy are now available with many receiving approval as first-line agents. These intraocular agents have shown benefits in reducing oedema and improving vision

and include ranibizumab, aflibercept, bevacizumab, dexamethasone and flucinolone.

Ranibizumab (RZB) is an anti-VEGF agent that has been shown to reduce DMO and deliver sustainable visual improvement.[44] The Ranibizumab for Edema of the mAcula in Diabetes-2 (READ-2) study demonstrated a significant improvement in VA compared to laser alone[45] and this improvement was sustained for up to three years with repeated injections.[46] The RESOLVE study confirmed the advantage of RZB over sham injections.[47] Further studies, such as the DRCR.net (Protocol I), RESTORE, REVEAL, RIDE and RISE have shown RBZ to be superior to laser, triamcinolone and sham treatments with early treatment demonstrating greater improvement in VA.[48-51] The LUCIDATE study demonstrated improvement in microperimetry and electrophysiology outcomes when treating DMO with RBZ.[52]

Aflibercept (AFL) is an anti-VEGF agent that binds all isoforms of VEGF.[44] The DA VINCI study demonstrated AFL to be significantly better than laser in the treatment of DMO.[53] The VIVID and VISTA confirmed these findings, as well as demonstrating that eight weekly injections were statistically as efficacious as four weekly injections.[54]

Bevacizumab (BZB) is another anti-VEGF agent that binds all isoforms of VEGF. Randomised controlled trials, such as DRCR.net (Protocol H) and BOLT, have demonstrated BZB to be superior to sham treatment or laser in the treatment of DMO.[55,56] However, unlike RZB and AFL, BZB does not have approval for use in treatment of DMO and so is used off-label, predominantly due to it being substantially cheaper than RZB and AFL.[44]

One disadvantage of anti-VEGF agents is the need for treatment on a monthly or two-monthly basis. Use of a single dexamethasone implant has shown to be effective for up to six months.[44] Prospective studies have shown dexamethasone to be significantly better than sham or laser treatment in both VA and in anatomical improvement.[57,58] The BEVORDEX study identified similar rates of improvement in VA between dexamethasone implant and BZB.[59] The MEAD study demonstrated a mean of only 4.1 injections were required over three years; however over half of

the patients underwent cataract surgery and quarter developed secondary ocular hypertension.[60]

Another corticosteroid implant that is approved for use in the treatment of DMO is flucinolone acetonide. The FAMOUS study demonstrated improvement in VA in persistent DMO that was sustained for over one year.[61] The FAME A and FAME B studies demonstrated a significant improvement in VA compared to sham injections, lower rates of glaucoma as compared to dexamethasone and improvement in VA in patients with chronic DMO (≥3 year duration).[62]

Newer treatments offer more hope to patients in maintaining, or even improving, VA in the presence of DMO. However this requires regular clinical appointments and carries risk of complications, and some patients are still at risk of losing vision.

2.8 The neurodegenerative theory of diabetic maculopathy

As described above the microvascular theory is commonly accepted as the most significant factor behind the visual loss associated with diabetic maculopathy. However over 50 years ago post-mortem studies of human eyes described degeneration of retinal ganglion cells and other neurons prior to the onset of vascular lesions.[63] In addition, atrophy of the inner retina has been described, attributed predominantly to the reduction in the number of ganglion cells in addition to the number of amacrine, horizontal, Müller and photoreceptor cells.[31,33] FA has not been useful in identifying these features, though the improved resolution of newer OCT systems has demonstrated inner retinal thinning in patients with DM but no maculopathy.[64]

Therefore it is possible that patients with DM develop sub-clinical retinal changes, i.e. changes within the neuronal network which clinicians are unable to detect. This suggests that deficits in visual function may occur before the patient notices any changes in their daily life. If we, as clinicians, are able to identify these sub-clinical deficits then it may be possible to identify those patients who are at most risk of developing visual loss. This would allow for early and targeted treatment for this 'at-

risk' group. Ultimately we may be able to reduce the rates of visual loss in patients with DM.

2.9 Discussion

In summary diabetic maculopathy is a significant cause of visual loss with obvious impact on an individuals' physical and mental well-being. As our understanding of the pathology behind diabetic maculopathy improves, newer treatment solutions are being investigated. However current treatment is based on preserving vision with many individuals continuing to lose vision. The identification of neurodegeneration of the retina prior to the development of microvasculopathy has been described previously and newer investigative techniques have made it possible to study the macula. By assessing the neuronal function of the macula in diabetic maculopathy it may be possible to determine severity of dysfunction. By correlating dysfunction to clinical severity it could be possible to identify subjects at risk of developing visual impairment and therefore be targeted for early intervention. In Chapter 3 I review the literature on current clinical techniques in the assessment of diabetic maculopathy and the current understanding of functional assessments of the macula in diabetes mellitus.

2.10 Aims of thesis

My aim is to correlate macular function to severity of diabetic maculopathy, and determine the potential role of functional investigations in the assessment of diabetic maculopathy. I will achieve this by assessing macular function using both objective (multifocal electroretinogram and oscillatory potentials) and subjective investigations (contrast sensitivity and microperimetry) to determine whether increasing severity of diabetic maculopathy is associated with decreasing macular function. I will compare these investigations to current clinical investigations (visual acuity and optical coherence tomography) to determine whether the former investigations provide more information on the state of retinal function before visual function is significantly reduced.

My secondary aim is to determine change in macular function longitudinally. I will achieve this by using the above investigations to assess whether retinal function decreases over time, and whether increasing severity of disease is associated with a greater decrease in retinal function. I will also assess whether these investigations may provide further information to identify those patients more likely to require treatment.

I hypothesise that increasing severity of diabetic maculopathy is associated with decreasing retinal function and that these investigations may help identify those with reduced function prior to development of visual impairment. I also hypothesise that subjects with more severe diabetic maculopathy will develop a greater reduction in retinal function than those with early or no diabetic maculopathy.

Chapter 3 Literature review

In this chapter I will review the key literature on the epidemiology, diagnosis and management of diabetic maculopathy. Using PubMed I searched for terms related to diabetes mellitus (DM), in particular diabetic maculopathy. These terms included:

- diabetic macular oedema (DMO)
- clinically significant macular oedema (CSMO)
- exudates in diabetic maculopathy
- ischaemic maculopathy
- enlarged foveal avascular zone (FAZ)
- hypertension in DM
- hyperlipidaemia in DM
- contrast sensitivity in DM
- fluorescein angiography in DM
- optical coherence tomography in DM
- multifocal electroretinogram in DM
- microperimetry in DM
- oscillatory potential in DM

From these searches, I selected landmark studies that have shaped our understanding of diabetic maculopathy. I have also selected papers that have been frequently referenced or whose results are comparable to my aims. I have critically appraised these papers, highlighted their strengths and weaknesses and then, for each of the following sections (and sub-sections), I have explained how I plan to incorporate them into my study to improve the strength of my results. Finally, I set the scene for the aims of my study.

3.1 Landmark studies in diabetic maculopathy

3.1.1 The Diabetic Retinopathy Study (DRS)

In the 1970's a randomised, controlled, prospective trial evaluated the efficacy of laser photocoagulation in the treatment of proliferative diabetic retinopathy (PDR).[65] In a three year period 1758 patients were recruited and randomised to either no treatment or treatment; the latter group were further randomised to treatment with either the argon or xenon laser photocoagulator. Patients were followed on a 4 monthly basis. The primary outcome was the risk of developing severe visual loss (SVL, Snellen acuity ≤5/200). At 1 year the treated group demonstrated a statistically significant reduction in risk of developing severe visual loss as compared to the control group (2.3% vs 3.6%, z=2.2). By 6 years the treatment group were less than half as likely to develop severe visual loss (16.6% vs 36.7%, z=9.0).

The DRS identified that a proportion of patients developed SVL despite laser treatment with diabetic maculopathy being identified as one of the factors. The authors proposed a further trial to determine the role of photocoagulation in the management of diabetic maculopathy – the Early Treatment Diabetic Retinopathy Study (ETDRS).

3.1.2 The Early Treatment Diabetic Retinopathy Study (ETDRS)

Between 1980 and 1985 this multicentre, randomised trial was designed with three aims in mind, one of which was to establish the efficacy of photocoagulation in the treatment of DMO.[42] A total of 3928 patients were recruited. Patients with macular oedema were assigned to either immediate or deferred retinal laser; those assigned to immediate photocoagulation were further randomised to either immediate macular laser or peripheral retinal laser. A broad range of features was used to determine the presence of maculopathy - from a few small hard exudates within a disc diameter of the centre of the fovea to the presence of extensive cystoid changes, regardless of visual acuity.[42]

All patients underwent visual acuity (VA) testing using a specifically designed chart, the ETDRS chart. Visual function was also assessed using the Farnsworth-Munsell

100-hue test, which analyses an individual's ability to differentiate between subtle changes in colour, and Goldmann perimetry, for the assessment of peripheral visual field.

Visits were scheduled for six weeks after treatment and then at four monthly intervals. Structural assessments were carried out through fundus photographs and fluorescein angiograms at specified intervals, and if clinically needed. The primary outcome was the percentage of patients who lost ≥15 letters on the ETDRS chart. A two-sample t-test was used to assess effects of photocoagulation between the two groups. A Z value of ±1.96 is equal to a p value of 0.05; a Z value of ±2.58 or greater corresponds to a p value of ≤0.01.

In Report 1, eyes treated with immediate macular laser (n=754) were compared to eyes in which laser was deferred (n=1490).[42] Those that underwent immediate macular laser were half as likely to lose ≥15 letters of vision at three years (12% vs 24%, Z >2.58). Subgroup analysis identified that eyes with a vision less than 70 letters (6/12) showed a gain of six letters or more in nearly 40% of eyes undergoing immediate laser as compared to 20% of those in whom laser was deferred (Z >1.96). Though eyes with a visual acuity worse than 60 letters (6/18) showed a reduction in loss of ≥15 letters, this was not statistically significant at three years (Z <1.96). Also, eyes with a visual acuity between 90 and 100 letters (20/15 or better) showed no significant difference in worsening by ≥15 letters between the two groups. In summary, the authors concluded that laser photocoagulation conferred benefit in a select group of patients. However, they also noted that, despite treatment, vision remained reduced in a significant proportion of patients.

Along with demonstrating the benefits of laser photocoagulation, the ETDRS provided the clinician with a method for determining severity of diabetic macular oedema and thus guidelines for laser application. As described in Chapter 2, diabetic maculopathy comprises of the presence of macular oedema, with or without exudates, and microvascular changes. The ETDRS attempted to determine which patients with diabetic maculopathy would gain the most from macular laser. The authors reported that a subset of subjects with certain features appeared to

gain the most benefit from macular laser and introduced the term 'clinically significant macular oedema (CSMO)'.

Features of Clinically Significant Macular Oedema (CSMO)[66]
• Thickening of the retina at or within 500 microns of the centre of the macula • Hard exudates at or within 500 microns of the centre of the macula, if associated with thickening of adjacent retina (not residual hard exudates remaining after disappearance of retinal thickening) • A zone or zones of retinal thickening 1 disc area or larger, any part of which is within 1 disc diameter of the centre of the macula

At baseline, CSMO was present in 1287 eyes and absent in 822 eyes. Eyes with these features had a statistically significant reduction in risk of developing visual loss of ≥15 letters following immediate focal laser as compared to deferred laser (~5% vs ~11% at 1 year, and ~10% vs ~30% at 3 years, Z≥3.29).[42] In comparison there was no statistically significant difference in development of visual loss of ≥15 letters between immediate laser and deferred laser in patients without CSMO at baseline (Z<1.96). Even with the exclusion of data from one centre the findings were similar.[66]

Patients were graded for presence or absence of retinal thickening at the centre of the macula at three years. Of those diagnosed with CSMO at baseline, 24% of patients (n=123) who underwent immediate laser demonstrated the presence of thickening as compared to 54% of patients (n=221) in whom laser was deferred.[66] This was statistically significant (Z≥2.58). Of those without CSMO at baseline, retinal thickening at the centre of the macula was detected in 16% of those undergoing immediate laser (n=81) and 25% those in the deferred laser group (n=173). This was not deemed to be statistically significant (Z<2.58).

The authors suggested that laser should be performed once CSMO is detected. However the authors noted that some patients continued to develop severe visual loss and some were at risk of developing CSMO despite adequate laser. However no explanation was provided for continuing visual loss or development of CSMO

despite treatment. Likely causes included progression of disease, adverse effect of laser, and longstanding retinal damage resulting in atrophy of retinal structures.

One limitation of the ETDRS is the lack of demographic data published in the initial reports. Though a subsequent report[67] provided baseline data comparing patients who received mild laser treatment and those who received full treatment, only VA was available for other inter-group comparisons. No multivariate analysis was performed to identify possible risk factors for worse prognosis. Nevertheless, this pivotal study provided sound evidence that improved understanding and treatment of diabetic maculopathy and continues to be referred to for the clinical management of diabetic maculopathy.

3.1.3 The Wisconsin Epidemiological Study of Diabetic Retinopathy

The Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) recruited 9283 patients with DM between 1980 and 1982 into a multicentre, prospective, observational study. Their aim was to determine the incidence of diabetic eye changes over specified time periods. Out of a sample population of 2990 patients with diabetes, there were 1426 subjects with no DMO at baseline and considered to be at risk of developing DMO. Macular oedema was defined as the presence of retinal thickening ≤ 1 disc diameter of the centre of the macula or the presence of photocoagulation scars in the macula as seen on stereoscopic fundus photographs. However no explanation was provided as to why these individuals were considered to be at risk.

The authors reported on the four year results in 1989.[68] 8.2% of patients with type 1 DM developed macular oedema, half of whom were diagnosed with CSMO. 5.2% of patients with type 2 DM developed macular oedema, with just over half identified as CSMO. The authors reported an increased incidence of DMO with increasing age in patients diagnosed with type 1 DM ($p<0.005$); no trend was found in patients with type 2 DM. An increased duration of DM was also associated with an increased incidence of DMO in type 1 but not type 2 DM ($p<0.001$ and $p=0.17$, respectively). Increasing severity of retinopathy was associated with a significantly increased risk of developing macular oedema in both groups of patients ($p<0.0001$). Other risk

factors identified for the development of DMO in patients with type 1 DM were elevated serum glycated haemoglobin (HbA1c, p<0.0001), higher diastolic blood pressure (p<0.01), current or previous smoking history (p<0.05), a history of cardiovascular disease (p<0.05), and a higher frequency of oral aspirin use (p<0.005). In patients with type 2 DM, a younger age at diagnosis of DM and a higher level of HbA1c were significantly associated with an increased incidence of DMO (p=0.0005 and p<0.0001 respectively). In neither group was systolic blood pressure or proteinuria shown to be significant. Sub-group analysis of those not on anti-hypertensive agents demonstrated only higher diastolic blood pressure to be significantly associated with an increased incidence of DMO in both groups (p=0.01 and 0.02, respectively).

In the 10 year report, the authors described an incidence of macular oedema and CSMO in 20.1% (130/688) and 13.6% (86/688) of patients with type 1 DM, respectively; in the patients with type 2 DM, the incidence was 18.6% (97/773) and 12.6% (63/773) respectively.[69] In patients with type 2 DM, those who were on insulin were more likely to develop macular oedema and CSMO (25.4% and 17.6% respectively) as compared to those treated without insulin (13.9% and 9.2% respectively). Duration of diabetes, severity of retinopathy, elevated diastolic blood pressure, and elevated HbA1c remained significant risk factors for both types of DM. In addition proteinuria and younger age at diagnosis were risk factors in patients with type 1 and type 2 DM respectively.

A multivariate analysis was performed using discrete linear logistic regression.[69] In patients with type 1 DM, an increase in HbA1c by 1% was associated with a 56% increased risk of developing macular oedema (OR 1.56; 95% CI 1.38-1.76); an increase in HbA1c by 1% point between baseline and year 4 was associated with a 37% increased risk (OR 1.37; 95% CI 1.23-1.52); an increase in severity retinopathy by one step was associated with 12% increased risk (OR 1.12; 95% CI 1.05-1.19); and a duration of diabetes at baseline up to 17.1 years was associated with a 6% increased risk (OR 1.06; 95% CI 1.01-1.12). However, duration of DM of 25 years at baseline was associated with a reduced incidence of macular oedema (OR 0.92; 95% CI 0.87-0.98).

In patients with type 2 DM,[69] an increase in HbA1c by 1% was associated with a 65% increased risk of developing DMO (OR 1.65; 95% CI 1.42-1.92); an increase in HbA1c by 1% from baseline to year 4 was associated with 18% increased incidence of DMO (OR 1.18; 95% CI 1.03-1.34); being female was associated with a 74% increased incidence of DMO (OR 1.74; 95% CI 1.05-2.87); increased severity of retinopathy by one step was associated an increased incidence (OR 1.18; 95% CI 1.10-1.26); and an increase in diastolic BP by 10mmHg was associated with a 35% increased incidence (OR 1.35; 95% CI 1.10-1.66).

However, WESDR may have underestimated the incidence of DMO as it only reported findings at a specific time points and not development of macular oedema at any point during the four years. No analysis was performed to assess risk factors for developing CSMO or to correct for confounders nor was there any evaluation of VA during the study. To minimise the effect of confounding in my study I aimed to match recruits for age and correct for blood pressure, duration of DM, HbA1c and lipid profile.

The management of DM has altered considerably since these studies were undertaken. In addition, due to the population mix, these results may not be applicable to other populations. Nevertheless, like the ETDRS, WESDR has been a pivotal study in our understanding of diabetic maculopathy. It helped identify the risk factors for development of DMO, factors that the clinician still assesses to this day in the management of diabetic maculopathy.

3.2 Longitudinal studies of diabetic maculopathy

As described above, the WESDR established rates of development of DMO and CSMO and associated risk factors. The modification of systemic risk factors, such as HbA1c, lipid profile and BP, remain an essential component in the management of diabetic maculopathy. I explore these risk factors further in this chapter.

The Los Angeles Latino Eye Study was a longitudinal, observational study of a Latino population with DM.[70] The authors aimed to demonstrate the incidence of DR and diabetic maculopathy after four years. Out of 775 participants who completed four

years of follow up, the overall incidence of macular oedema (either retinal thickening within 1 disc diameter of the fovea or presence of macular laser) was 5.4% and of CSMO was 7.2%. The study only found increasing duration of DM to be a significant factor in development of maculopathy. The authors reported that 11.1% of those with a duration of DM greater than 15 years developed macular oedema as compared to only 5.1% of those with a duration less than 5 years (p=0.003). The equivalent values for development of CSMO is 9.1% in those with duration greater than 15 years compared to 5.1% with duration less than 5 years (p=0.002). The authors did not look at incidence of development of exudates as a marker of macular oedema which I have included in my description of maculopathy. Also their data is limited to a certain ethnic group that is different to our local population and so may not be applicable to other populations.

Early retinal changes have been studied that might predict the subsequent onset of diabetic retinopathy though the results have been inconsistent. One small retrospective study of patients who developed visual loss secondary to diabetic maculopathy had their screening images reviewed.[71] Patients were selected if colour images demonstrating stable background diabetic retinopathy (BDR) for at least one year were found. For each study patient three controls were selected matched for retinopathy grade, age at onset of diabetes and duration of diabetes. There were 11 study patients and 33 controls. Retinal findings were graded by a computer according to total number of haemorrhages and exudates within the macula and their location with respect to the centre of the fovea. The study identified that those developing vision loss secondary to maculopathy had a significantly greater number and coverage of haemorrhages and exudates involving and temporal to the fovea. Limitations of this study include small number of subjects included in the study, being retrospective, lack of data on systemic risk factors and the onerous method of quantifying haemorrhages and exudates which is not practical in routine clinical practice.

In my study I will record those risk factors associated with the development of diabetic maculopathy, namely age, duration of DM, severity of retinopathy, HbA1c, lipid profile, BP, smoking status, and systemic treatment (use of insulin, oral

hypertensive and oral lipid-lowering agents). Where appropriate I will correct for these factors during data analysis.

3.3 Current clinical assessment of diabetic maculopathy

Clinical examination remains the accepted standard in the assessment of diabetic maculopathy. In the following sections I will describe the benefits and drawbacks of each technique used in the assessment of diabetic maculopathy.

3.3.1 Visual acuity

Visual acuity (VA) is a subjective test of ocular function. It is assessed by the subject reading a chart at a specified distance. The chart is comprised of letters of specified sizes, and results recorded as without use of refractive correction (unaided VA), with use of subjects own glasses or a pinhole (VA with glasses/PH) or after refraction (best corrected visual acuity, BCVA).

As the most commonly used measure of the health of the eye, a reduction in VA represents a red flag sign for the health professional. As seen in the preceding paragraphs, visual loss was used as the main outcome in ETDRS for determining response to treatment. However VA appears to be insensitive to early changes of DR.

In a retrospective study of 103 eyes with diabetic maculopathy the authors concluded that VA was a poor predictor for the presence or absence of clinically significant diabetic maculopathy.[72] The diagnosis of diabetic maculopathy was made on findings from clinical examination and divided into sub-types using FA for guidance. Overall 68% of eyes demonstrated a visual acuity of 6/12 or better despite the presence of retinal thickening ± exudates within 1 disc diameter of the fovea. Despite macular laser treatment 34.1% of treated eyes suffered worsening of vision. This was a retrospective study and, apart from HbA1c, it did not assess any of the systemic markers. The authors aimed to correlate vision to type of maculopathy, rather than determine severity of maculopathy such as CSMO. The follow up period was short (6 months) and so the findings cannot be used to

estimate progression of maculopathy. I will look at VA in relation to the different severities of maculopathy and progression to worsening or laser.

A retrospective cross-sectional study of 1549 subjects with DM reported that diabetic maculopathy accounted for only 15% of cases of VA worse than 6/18.[73] The authors concluded that VA is not a reliable criterion in predicting STDR with the majority of visual loss attributable to causes other than DR. However, these subjects had their VA tested with only glasses or contact lens correction rather than a full refraction. Therefore, it is possible that the reduction in VA may have been attributed to causes which could be corrected through refraction rather than diabetic maculopathy.

In a prospective study of 59 eyes of patients, BCVA was assessed in the presence of varying severities of DR.[74] Twenty subjects were deemed to have macular oedema, defined as the presence of any retinal thickening within the macula based on stereo fundus photographs. The authors reported that BCVA was not significantly worse in subjects with macular oedema compared to those without oedema (p=0.90). However VA was significantly worse in subjects who had oedema at the centre of the fovea (p=0.006). The authors concluded that VA would not be a good prognostic indicator to determine those who are likely to develop diabetic maculopathy, but would probably aid in determining severity of disease.

In my study I will record BCVA using the ETDRS letter score as used in landmark studies such as the ETDRS and Diabetic Retinopathy Clinical Research Network (DRCR.Net). I will compare VA between different severities of maculopathy and to the functional investigations used in this study. I will exclude subjects who have reduced VA attributable to causes other than DR.

3.3.2 Contrast sensitivity

Contrast sensitivity (CS) is a specific measure of visual function that assesses the ability to distinguish an object from its background. Standard visual acuity charts present black letters on a white background. However, daily life comprises of objects of different shades against various background. Thus CS charts are comprised of target objects that reduce in contrast gradually, such as the Pelli-

Robson chart. This chart comprises of a series of capital letters of equal size that are arranged in triplets and gradually fade. The chart is read at 1 metre until there are 2 or 3 mistakes within a triplet.

Reduction in CS was first described in subjects with DR in 1982.[75] Since then several studies have described reduced CS in the presence of DR compared to those without DR in subjects with type 1 and type 2 DM[76-79] and in subjects without DR as compared to healthy controls.[80]

However there have been few studies that have reported changes in CS in the presence of diabetic maculopathy. In a prospective study[81] of 20 subjects with DM and 20 healthy controls, static CS was assessed in relation to features of ischaemic maculopathy on FA, namely perifoveal intercapillary area (PIA) and greatest linear diameter of foveal avascular zone (FAZ). All subjects had normal VA and none demonstrated the presence of either macular oedema on clinical examination or leakage on FA. Increasing PIA and increasing FAZ were correlated to significantly decreased CS ($p=0.016$, $r=-0.54$ and $p=0.005$, $r=-0.6$, respectively). Interestingly decrease in contrast sensitivity in subjects with DM was only observed at spatial frequencies of 6 and 12 cycles/degree, but not at 3 or 18 cycles/degree. The authors were unable to explain why there is such specificity though Sokol et al (1985)[82] reported largest loss in contrast sensitivity in subjects with DM occurred at 11.4c/deg. Another study reported decreased contrast sensitivity at 12 and 18 c/deg.[83]

Talwar et al (2001) prospectively assessed CS after focal macular laser treatment in subjects with CSMO.[84] The authors reported an improvement in CS after laser treatment, with a corresponding improvement in maculopathy, in 9/14 cases and concluded that CS offered an alternative method for assessing retinal function after treatment. Improvement after treatment suggests that CS is reduced in the presence of CSMO. However there was no control group to determine whether difference in CS is due to maculopathy or due to inter-subject variability.

However there are no studies that have looked at CS with respect to different severities of diabetic maculopathy. I will analyse CS and compare it to BCVA to

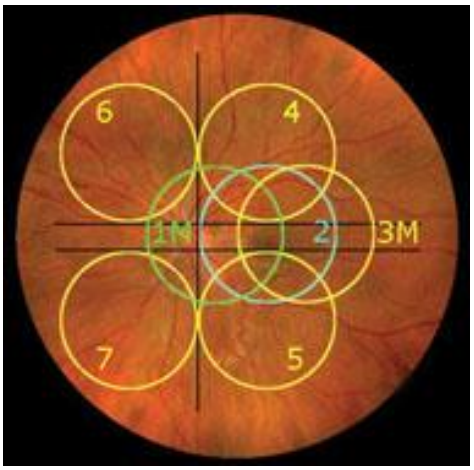
determine if this assessment of visual function is better suited to determining severity of disease.

3.3.3 Colour photographs

Colour photographs (CF) were used in the DRS and ETDRS to capture images of the retina for grading of retinopathy.[67,85] The authors utilised a seven-field stereo image schematic (Figure 3.1).

There are several advantages of taking photographs for grading of retinopathy. It allows for objective monitoring of disease progression, rather than relying upon the subjective assessment of clinical examination, provides time for health professionals to evaluate the fundus, acts as a permanent record, and can be used for teaching and quality assurance.

Figure 3.1: Schematic representation of the seven field fundus photographs showing the areas captured



Each field covers a diameter of 30^{o} and represent:

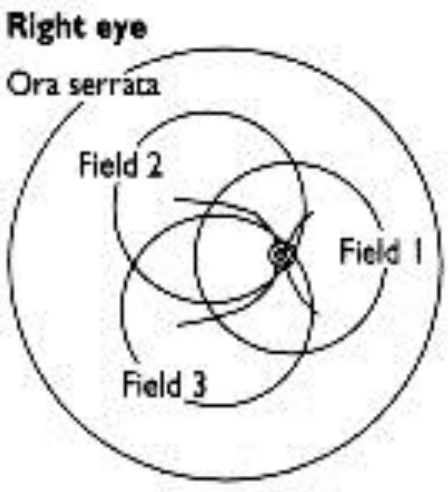
- Field 1M – Centred on the optic disc
- Field 2 – Macula centred
- Field 3M – Temporal to macula
- Field 4 – Superior temporal
- Field 6 – Superior Nasal

- Field 5 – Inferior temporal
- Field 7 – Inferior nasal

However patients found the seven-field photographs to be uncomfortable due to the intensity of the flash and the time taken to capture the images, especially with stereo pairs. Moss et al (1989) compared 2, 3 and 4-field images to determine adequacy of grading retinopathy.[86] Two-field photographs consisted of images F1 and F2; 3-field consisted of images F1, F2 and F4; and 4-field photographs consisted of images F1, F2, F4 and either F3 or F5. Of the original cohort of subjects included in WESDR, images of 2410 eyes were included in the analysis. The overall agreement to the 7-field photographs was 80-85% for 2-field images; 87-93% for 3-field images; and 91-95% for 4-field images. The authors also noted that for severe retinopathy there was a risk that patients may be assigned a lower grading with fewer image fields than the grade assigned with 7-field images. The authors concluded that the use of fewer fields may be adequate for some studies, but a cut-off grade would need to be determined beyond which more fields would need to be included in grading.

In the Liverpool Diabetic Eye Study, 295 subjects underwent three overlapping 45^{o} images of the fundus (Figure 3.2) and direct ophthalmoscopy to detect the presence of STDR.[87] Slit lamp biomicroscopy by a medical retina specialist was used as the reference standard.

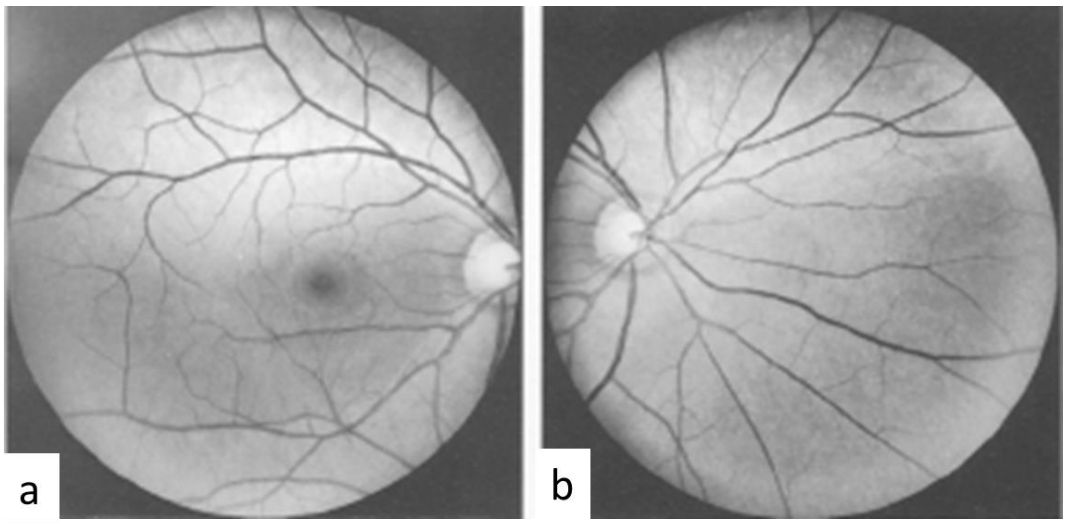
Figure 3.2: Schematic of the three 45^{o} fields used in the Liverpool Diabetic Eye Study[68]



The authors reported high sensitivity (89%) for the detection of STDR. However, compared to the reference standard, photography missed five subjects with STDR, all with maculopathy. The positive predictive value for photography was 86%, but this fell to 51% if images that were not gradeable or obtainable were included in the analysis, with media opacity being implicated in the majority of these cases. The authors concluded that a community based photographic screening programme would be effective in detecting sight-threatening diabetic eye disease.

The European Community funded Concerted Action Programme into the epidemiology and prevention of diabetes (EURODIAB) IDDM Complications Study used a 45^{o} camera to take two images of the retina: i) the centre of the optic disc lies at the nasal edge of the image to view the macula and the temporal retina (Figure 3.3a); ii) the optic disc is located one disc diameter from the temporal edge of the image to view the nasal retina (Figure 3.3b).[88]

Figure 3.3: Images of the 45^{o} field taken in the EURODIAB IDDM Complications Study; a) macular field b) disc/nasal field[88]



The authors compared detection of retinopathy between 2-field image sets and 7-field image sets and reported 100% agreement on detection of retinopathy between the two methods. For grading severity of retinopathy, at least one grader matched verified results in 93% of eyes, and three or more graders matched in 76% of eyes. There was good intra- and inter-observer agreement for the 2-field images

(κ=0.85 and 0.83, respectively). The authors concluded that 2-field photography offered a comparatively simple method for assessment of diabetic retinopathy but also stated that the lack of stereoscopic images meant that maculopathy could not be fully assessed.

Scanlon et al (2003) compared ophthalmologist examination using slit lamp biomicroscopy, 7-field 30^{o} photographic imaging, and 2-field 45^{o} photographic imaging in the screening of diabetic retinopathy.[89] Compared to clinical examination, 2-field photography gave a sensitivity of 82.8% and specificity of 92.9% while 7-field photography gave a sensitivity of 96.4% and specificity of 82.9%. However 15.3% of 7-field images and 1.5% of 2-field images were ungradeable compared to none of the eyes examined clinically. The authors concluded that clinical examination by an experienced retinal specialist compared favourably to 7-field photography but suffers from difficulty of inter-observer comparisons. Seven-field photography had a high technical failure rate.

In the early 21^{st} Century, following on from the establishment of several local population-based screening programmes for DR, the National Screening Programme (NSP) for diabetic eye disease was developed.[90] The aim of the programme was to detect sight-threatening disease early enough so treatment could be initiated promptly to prevent visual loss. Within the programme, a minimum of two 45^{o} or 50^{o} fields of the retina were to be taken. The grading system used is summarised in Table 3.1.

Once the images are reviewed by a trained person, a grade is scored for each category. Therefore, each eye is scored as R 0/1/2/3, M 0/1, P 0/1. If images are of too poor a quality to grade or were not able to be obtained, then a grade of 'U' is given. Patients who score R≥2, M1 or U, or are found to have other lesions, are referred to the hospital eye service.

Table 3.1: Grading protocol used by the NSP. Haem = haemorrhage; IRMA = intraretinal microvascular abnormality; CWS = cotton wool spots; TRD = tractional retinal detachment; DD = disc diameter[90]

Grading	Level	Diagnosis	Features
Retinopathy (R)	0		None
	1	Background	Microaneurysms (MA) Retinal haem ± exudates
	2	Preproliferative	Venous beading Venous loop or reduplication IRMA Multiple deep, round or blot haem (CWS - search for above features)
	3	Proliferative	New vessels on disc (NVD) New vessels elsewhere (NVE) Preretinal or vitreous haemorrhage Preretinal fibrosis ± TRD
Maculopathy (M)	0		None
	1		Exudate within 1 DD of centre of fovea Circinate/ group of exudates within macula Retinal thickening within 1 DD of centre of fovea (if stereo available) Any MA or haem within 1 DD of centre of fovea only if associated with a best VA of < (if no stereo) 6/12
Photocoagulation (P)	0		None
	1		Focal/grid to macula
Ungradeable/ Unobtainable (U)			Peripheral scatter Poor view Unscreenable

In my study I will perform slit lamp biomicroscopy to grade DR and to assess for maculopathy. I will also perform non-stereoscopic 7-field retinal photography so that a digital record of retinopathy status is available for any disagreements with retinopathy grading. I will grade retinopathy as per the NSP grading protocol in Table 3.1. Patients with a retinopathy grading of R3 will not be included due to their need for urgent photocoagulation.

3.3.4 Fluorescein angiography

As described earlier, DM is associated with vascular changes. Clinical examination allows for visualisation of some of the pathological changes. However early changes may only be subtle and so not seen on slit lamp biomicroscopy. Fluorescein angiography (FA) allows for indirect visualisation of retinal capillaries and is an invasive investigative tool consisting of the injection of a dye (fluorescein sodium 20%) into the venous circulation followed by the capture of retinal images. It is routinely used in clinical practice to:

- visualise the perifoveal capillary network and assess the FAZ
- identify the source of any leakage
- identify possible foci for performing laser treatment
- determine the presence of transudate at the fovea
- assess the perfusion of the peripheral retina

The ETDRS utilised FA for two main reasons: i) to guide treatment of macular oedema, and ii) to assess characteristics not well assessed by colour photography.[67] Accredited photographers captured stereoscopic fluorescein angiograms of seven 30^{o} fields based on the colour photography protocol described above. One of the fields (2F) was centred ½ disc diameter (DD) temporal to the foveal centre, where 1DD was equivalent to 1500µm. A standard protocol was described by the authors and consisted of:

i) Transit/early phase

- Rapid series of field 2F from 13 to 28 seconds after dye injection

- Selected depending on clinical judgement of investigator or randomly by birth month if both eyes were studied
- Allowed for assessment of perifoveal capillary net
- At least one stereoscopic pair of images were captured to show full capillary filling

ii) Mid-phase

- Lasted from 35 to 120 seconds after dye injection
- Non-rapid series – stereoscopic pair of field 2F as soon as possible after rapid series
- Stereoscopic pairs of field 1F to view this region

iii) Late phase

- Taken at 7 to 9 minutes
- Stereoscopic pairs of field 2F of each eye
- To document degree of late leakage of fluorescein

To assess for location of lesions with relation to the foveal centre, images of field 2F had a grid superimposed onto the left hand image of the stereoscopic pair. This grid consisted of three solid circles of the following radii from the centre of the macula:

- Innermost circle of 500µm – represents location of pathology to aid diagnosis of CSMO
- Middle (2^{nd}) circle of 1500µm – represents lesions within 1DD of the centre of the fovea
- Outer (3^{rd}) circle of 3000µm – represents the outer extent of the macula

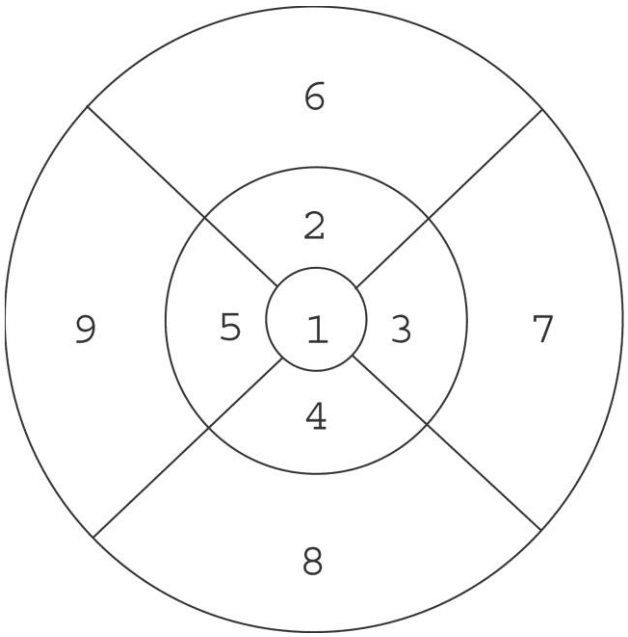
Images were then graded for disease severity. A grade was assigned depending upon extent of structural damage or comparison to standard photographs. Features that were assessed in the early and mid-phase included the size and outline of FAZ, loss of capillaries within the PIA, and narrowing, staining or loss of contour of arterioles within the macula. In the late phase the authors assessed for leakage, paying particular attention to source, severity and appearance of leakage.[67] The

standard photographs were selected from stereoscopic pairs of nine angiograms. They show specific subfields of the angiogram and define the boundaries between steps in the grading scale.[67]

In the ETDRS a grid was used to analyse the macula on both CF and FA (Figure 3.4).[67] It was centred on the fovea and comprised of three rings: the central subfield ring has a diameter of 1000 µm; the inner ring 3000 µm; and the outer ring 6000 µm. The inner and outer rings were further divided into 8 subfields with superior, nasal, inferior and temporal zones within each ring. A 10th subfield was occasionally used when assessing lesions temporal to the grid.

Grading of images was performed by one ophthalmologist and two graders who were trained by the ophthalmologist.[67] Following an initial grading exercise consisting of inter- and intragrader comparisons, the graders analysed a random sample of angiograms of 100 eyes. Intergrader comparison was performed looking at percentages of agreement and degrees of disagreement and calculating kappa statistics. Weights for kappa varied depending on the number of steps in the grading protocol, the "weighted Kappa statistic".

Figure 3.4: Example of the grid used in the ETDRS for assessment of the macula. 1= central subfield; 2-5 = inner ring; 6-9 = outer ring.



Results of intergrader comparison demonstrated severity of leakage was the most reproducible characteristic with 99% agreement within one step (κ=0.742, p=0.044). There was 'substantial agreement' in comparing cystoid spaces (κ=0.674, p=0.127). Other features, such as FAZ size, showed only fair to moderate agreement (κ ranged from 0.342 to 0.583, p>0.05). However only 100 eyes were graded this way and severe grades of certain features were infrequently seen. This subjective variation in the analysis of FA often results in variations in clinical practice.

In ETDRS report 19, the authors analysed FA features of diabetic maculopathy associated with poor visual outcome.[91] All patients in this report had diabetic macular oedema that questionably or definitely involved the centre of the macula. They compared immediate macular laser and deferred laser groups using a two-sample test and between the different severities of the baseline characteristics on FA using a Generalised Estimating Equation model. Z-values and their equivalent p values have been described in Section 3.1.2.[91]

The authors reported that outcomes of MVL, regardless of FA feature, were less favourable in subjects in whom laser was deferred compared to subjects assigned to immediate focal laser. However the results are complicated by patients receiving focal laser during the study; at 5 years this occurred in 60.8% of those in whom laser was deferred at baseline and 75% of those who received immediate focal laser. Individuals also received peripheral scatter laser if required. These laser procedures are likely to affect visual acuity outcomes, either due to improvement in retinopathy, through laser applications involving the macula or worsening of DMO due to PRP.

The results presented only describe findings for one variable on FA. The authors did not present findings when correcting for confounders or combining different FA features. It is likely that individuals with two or more severe features are likely to have a poorer outcome, though we do not have data to confirm this. Other limitations include the difficulty in acquiring 7-field images, the photographer training required and the small field of view. Nevertheless, the ETDRS was pivotal in demonstrating the role of FA in assessing diabetic maculopathy. The use of

stereographic FA with a systematic grading protocol and accredited graders provided robust evidence and protocols that are used in research and clinical practice to this day.

A prospective analysis of perifoveal microcirculation on FA was performed comparing subjects with diabetic maculopathy and matched healthy controls.[92] The subjects with maculopathy were further subdivided into those with impaired VA of 6/15 or worse and those with normal VA; none had CSMO. Subjects with DM had significantly larger FAZ and PIA as compared to healthy controls ($p<0.001$). Subjects with reduced VA had a significantly increased FAZ ($p<0.01$) as compared to those with preserved VA. In addition VA was significantly correlated to FAZ ($r^2=0.51$). However systemic risk factors (blood pressure, cholesterol) were not analysed.

In my study all subjects will undergo FA to assess the greatest linear diameter (GLD) of the FAZ and assess for features of ischaemic maculopathy such as perifoveal capillary loss.

3.4 Optical coherence tomography

Optical coherence tomography (OCT) provides cross-sectional images of the retina with high reproducibility and high resolution.[93] The OCT platform I have utilised in the studies described in this thesis is a spectral-domain (SD) OCT which relies on alterations in light frequency to demonstrate the retinal structures. A light source emits an infrared beam which passes through a partially reflective mirror and is split into two beams. One travels straight through to the retina and the other to a fixed reference mirror. The reflected light from the retina and the reference mirror is then distributed by a dispersive element onto a detector strip according to their optical frequencies. The signal generated is processed to produce a greyscale image of the retina reflecting the different depths and density of structures within each layer.

Analysis of the OCT image consists of an ETDRS grid which I have described in Figure 3.4. Two main measurements are recorded from the central subfield: central point thickness (CPT) refers to the thickness of the retina at the centre of the fovea; mean

central subfield thickness (CSFT) refers to the mean thickness across the whole of the central ring. A DRCR.net study demonstrated a very high correlation (correlation coefficient = 0.99) between CPT and CSFT in patients with DMO.[94] However CPT measurements have been shown to vary greatly as compared to CSFT.[95] In addition patients with CSMO may demonstrate a relatively normal CPT if fluid is present at the edge of the central ring. Therefore CSFT has been more commonly utilised in assessing DMO in research and clinical practice.[93]

The reproducibility of OCT measurements has been demonstrated by several studies. In one small prospective study, 10 healthy volunteers and 10 patients with DMO underwent a series of radial OCT scans by two different examiners at one visit and then a further radial scan one week later.[96] In healthy subjects the repeatability coefficient was 14 µm at the macula and ≤7 µm in all other areas, with inter-visit reproducibility of ±5 %. In patients with diabetes, the repeatability co-efficient was 21 µm at the macula, with a reproducibility of ±6 %. The intraclass correlation coefficient was greater than 0.98 in all measurements for people with DM.

In a retrospective study of 56 eyes[97] of patients with DM with a follow up of 17 months and a baseline mean CSFT of 219µm, the CSFT varied by a median of 18µm. None of the eyes had macular oedema at baseline or at last follow up. Thus the authors determined that a change of 10% or more in macular thickness over time would be clinically significant.

The range of different OCT systems, such as time-domain (TD) and spectral-domain (SD), poses problems in comparing retinal thickness between them. SD-OCT systems generate significantly higher values for retinal thickness compared to TD-OCT.[93] This is primarily due to the structures used as reference points. TD-OCT measures retinal thickness from the inner limiting membrane (ILM) to the IS/OS junction of the photoreceptors; SD-OCT measures from the ILM to the retinal pigment epithelial (RPE). To avoid this discrepancy all OCT imaging will be performed on a single SD-OCT system.

3.4.1 OCT in detecting diabetic maculopathy

In diabetic eye disease OCT has been used to demonstrate the presence and morphology of microaneurysms,[98] diffuse DMO,[41] retinal thickening,[38] and retinal exudates as well as to monitor response to treatment.[35] It allows for rapid detection and quantification of DMO.[16] The main errors associated with OCT are based around the automated segmentation of the different retinal layers, substantial differences between platforms and misidentification of the foveal centre.[99,100]

Studies have been performed to compare current methods of assessing diabetic maculopathy (clinical examination, FA) to OCT in the detection of DMO. A prospective, double-masked study comparing clinical examination to OCT in the detection of macular thickening identified moderate agreement between the two methods (κ=0.63 for the fovea, and 0.36-0.42 for the parafoveal zones).[101] Disagreement between the two techniques was more likely to be due to detection of thickening on OCT not seen clinically (68% of disagreements). The authors concluded that OCT could complement or maybe even supersede clinical examination in the detection of DMO. However the clinical significance of these extra cases of increased thickness was not discussed. One cause of the disagreement could be the use of the 78D lens for clinical examination rather than a 60D lens or a contact lens, as used in the ETDRS, which could potentially underestimate the presence of macular thickening.

A retrospective study of 195 eyes of patients with varying stages of diabetes was performed to compare OCT features with clinical and FA findings.[38] CSMO was present in 57.4% of eyes and cystoid macular oedema (CMO) in 9.2%. There was no macular oedema in the remaining 33.3% of eyes. Retinal swelling was noted in 148 eyes (75.9%) on OCT, 36 of which were not seen clinically. OCT demonstrated serous retinal detachment in 19 eyes that were not detected clinically or by FA. Macular oedema was detected by OCT but not clinical examination in 22% of eyes and by clinical examination and not OCT in 1% of eyes. The study also identified different types of macular oedema with varying levels of central foveal thickness (CSFT): 'sponge like retinal swelling' in 129 eyes with a mean CSFT of 347±110µm; CMO, 23 eyes, 441±96µm; sponge like swelling plus serous retinal detachment, 12

eyes, 460±135µm; combination of all, 7 eyes, 566±189µm. There was a statistically significant difference between the groups (ANOVA $p<0.001$, Bonferroni correction, $p<0.05$ for all patterns). There was also a significant negative correlation between CSFT and VA (correlation co-efficient: -0.528, $p<0.01$). There was no significant difference in CSFT between patients with macular ischaemia and those without. There was a statistically significant increase in macular thickness with increasing severity of leakage on FA (Bonferroni correction, $p<0.05$). As this was a retrospective study it is possible that the presence of macular oedema on clinical examination was underestimated.

A prospective, cross-sectional study compared retinal thickness on OCT and fluorescein angiography findings in patients with macular oedema (n=136) and controls (n=30).[102] OCT was performed using a Zeiss-Humphrey OCT 2000 scanner and comprised of three horizontal and one vertical scan through the fovea. Retinal thickness was manually measured at the centre of the fovea and 0.5mm and 1.0mm from the centre of the fovea. Foveal thickness was found to be greater in patients with macular oedema as compared to controls (307±136µm vs 153±15µm, $p<0.001$). In patients without CSMO there was no statistically significant difference in retinal thickness (159±15µm) though there were only 20 eyes. There was an intermediate relationship between average retinal thickness and leakage on OCT ($r=0.44$, $p<0.00001$) though this was not compared to central foveal thickness. There was no correlation between retinal thickness and retinal non-perfusion ($r=0.004$, $p=0.96$). Sensitivity was 89% and specificity 96% in detecting CSMO by measuring foveal thickness. These results suggest the value of OCT in detecting CSMO and assessing diabetic macular oedema but not macular ischaemia. Also the strength of the relationship between thickness and leakage suggests OCT cannot be used for determining treatment of CSMO. This study used only a central foveal point to determine foveal thickness, whilst our OCT records central foveal thickness as a mean thickness covering the central subfield, the mean CSFT. Measurements were performed by a single individual, resulting in possible bias in data recorded.

Disruption of the photoreceptor layer in DMO has been associated with a reduced visual acuity. In a retrospective study of 62 eyes from 38 patients with DMO, the

inner segment/outer segment (IS/OS) junction over the central 1mm was assessed and correlated to visual acuity.[103] 38.7% of eyes did not demonstrate any disruption, whereas the remaining eyes had a mean percentage disruption of 26.9%. There was a statistically significant correlation between percent disruption and visual acuity (p=0.0312) and a regression coefficient of -0.3, i.e. for each 1% increase in disruption, vision decreased by 0.3 letters. However 69.8% of eyes had previously received treatment, including laser photocoagulation, which is known to disrupt the RPE and thus the photoreceptor layer. The authors reported a trend association (p=0.07) between macular volume and visual acuity; this suggested that retinal thickening may be associated with changes in visual acuity. No multivariate analyses were performed to determine the relation between IS/OS junction disruption and visual acuity adjusting for macular thickness.

In a retrospective case series, 15% of eyes with severe NPDR or PDR had retinal thickening on OCT not seen clinically.[104] This has been termed 'subclinical DMO' though its importance has not yet been determined.

3.4.2 Longitudinal studies of OCT in DMO

In a retrospective study of male patients with type 2 DM and subclinical DMO, defined as OCT measured thickness of 200-300µm on OCT in the absence of clinical signs suggestive of macular oedema, the authors aimed to determine progression to CSMO.[105] A control group was matched for age, sex, race and duration of DM and had an OCT thickness of <200µm. Of the 52 eyes with subclinical DMO, 16 (30.8%) progressed to CSMO as compared to 6 out of 72 eyes (8.3%) in the control group, though disappointingly statistical significance was not performed. Those who progressed to CSMO had a mean retinal thickness greater than those who did not, though statistical significance again was not provided. Multivariate analysis identified three risk factors for progression: prior history of CSMO (OR 3.69, CI 1.10 to 12.31, p=0.03); 10µm increase in retinal thickness (OR1.15, CI 1.03 to 1.28, p=0.01); and lower age (each 1 year increase was associated with OR 0.91, CI 0.84 to 0.98, p=0.01). The authors concluded that patients with subclinical DMO are at risk of developing CSMO but larger prospective studies were required. This study was retrospective and limited to male patients and so may not be fully applicable to

our clinical setting. Statistical significance was not calculated to compare subjects and controls and so the greater proportion of subjects developing CSMO may not be a statistically significant finding. More importantly there is no set definition of subclinical DMO and so it is difficult to compare different studies. In my thesis the diagnosis of severity of maculopathy is based on clinical examination and FA findings, as per current clinical standards, rather than OCT findings.

3.4.3 OCT and systemic risk factors

A prospective, cross-sectional study of patients with type 2 DM and mild or no DR looked at changes in macular thickness with respect to systemic risk factors.[64] Mild DR was defined as less than 5 haemorrhages or micraoaneurysms (HMA) on examination. BP, serum HbA1c, lipid profile, VA, duration of DM and insulin use were recorded. Six line scans of 6 mm with the midpoint centred on the fovea were taken. A total of 37 points on the OCT were measured: i) central foveal thickness (CFT) was an average of the measurements at the midpoint of the six line scans; ii) total foveal thickness (TFT) was a mean of the 12 points at 500µm from the centre of the fovea; iii) total macular thickness (TMT) was a mean of all values within the 6000µm ring excluding the CFT. Increased duration of diabetes was associated with a significantly decreased retinal thickness in patients with diabetes: CFT showed a regression coefficient of -0.28, p=0.003; TFT, r=-0.25, p=0.006; TMT, r=-0.25, p=0.003). There was no significant relationship between systemic HbA1c and the three macular thickness measurements (for all r= -0.07, p>0.46). There was no difference in thickness between patients with no DR and those with mild DR when adjusted for insulin use. Also there was no significant difference in retinal thickness between patients with DM and controls. The authors suggested that reduction in macular thickness with increasing duration of DM represented likely early retinal neurodegenerative changes. However, only a small subset of patients were included in the study (age between 60 and 75 years, type 2 DM, males >96%). So the results are not truly applicable to the general population.

3.4.4 OCT in absence of diabetic retinopathy

Similar to the study by Asefzadeh et al (2008),[64] a prospective study of patients with type 1 DM was performed.[106] The authors described no significant difference in

CSFT between patients with DM and controls. There was significant pericentral (i.e. the inner ring of the ETDRS grid) thinning in patients with mild DR (267±20µm) but not in those with no DR (276±14µm) as compared to controls (281±13µm, p=0.005, 95% CI -23.10 to -4.46). The authors attributed these changes to neurodegenerative changes associated with DM. However the authors did not adjust for duration of DM which was significantly different between the two groups.

A cross-sectional study of 39 subjects[31] with DM but no DR reported a significant reduction in CPT (168.64µm vs 177.74µm, p=0.012) and mean photoreceptor layer thickness (PLT) (61.62µm vs 68.79µm, p<0.0001) compared to a group of healthy controls. Sub-analysis found decrease in CPT was only significant in those of age < 50. Mean PLT was significantly reduced irrespective of age in patients with DM. Increased duration of DM was associated with decreased PLT but not CPT. Elevated HbA1c did not appear to influence CPT or PLT. This study only included patients above the age of 40. CPT was manually measured by a single grader; no inter- or intra-observer analysis was performed. Other systemic factors such as blood pressure and lipid profile were not recorded. No adjustment for age or duration of DM was made in the analysis of macular thickness.

A similar study comparing OCT macular thickness between diabetic patients without DR and controls was performed with a different methodology.[107] A single horizontal and a single vertical scan of 3 mm were performed through the foveal centre. Mean thickness was calculated along these lines to give mean retinal thickness. The authors reported a non-significant increased mean retinal thickness in patients with diabetes compared to controls (237.7µm vs 230.0µm, p>0.05). Sub-analysis revealed the superior retina to be significantly thickened in patients with diabetes. These results are in contrast to the study above.[31] As the mean age was similar between the two studies it is likely that the difference may be due to differences in methods of calculating thickness, i.e. calculating the mean thickness across the central 3000 µm of the macula. In addition to relatively small numbers in both groups (n=34 and 32 in diabetics and controls, respectively), the study is limited to patients with type 2 DM.

3.4.5 OCT and VA

Several studies have attempted to relate foveal thickness to VA.[108-111] In a retrospective study of 27 subjects with CSMO[108] there was moderate correlation between BCVA on Snellen chart and macular thickness (r=0.55, p=0.0027); however this study was limited to patients below the age of 40 with advanced maculopathy.

In a prospective study comparing logarithm of minimal angle of resolution (logMAR) BCVA to CPT in patients with maculopathy[111], the authors reported a significant decrease in BCVA with increasing CPT in patients with CSMO (r=0.640, p=0.0013); however the authors did not report their findings in patients with maculopathy without CSMO or healthy controls.

In a prospective study of 251 eyes with definite increased retinal thickness on clinical examination, there was a moderate correlation between ETDRS protocol BCVA and CPT on OCT (r=0.52).[109] The authors reported a 4.4 letter decrease in BCVA for every 100 µm increase in CPT (CI 3.5-5.3, p<0.001) following macular laser treatment. A multivariate analysis estimated a decrease of 3.6 letters (p<0.001). Following macular laser treatment a subset of eyes (17%) demonstrated a paradoxical improvement in BCVA with increasing CPT and 26% of eyes demonstrated worsening BCVA despite improvement in CPT. Once again this study focussed on individuals with CSMO with no data presented on non-CSMO maculopathy.

In a prospective study of 62 eyes, CSFT was compared to ETDRS protocol BCVA in subjects with CSMO.[110] The authors reported a moderate correlation between CSFT and BCVA only where thickening involved the central 1000 µm zone of the macula.

Therefore OCT measured thickness appears to only be correlated to VA when severe disease is present and involves the central macula. Therefore, in early stages of diabetic maculopathy, OCT appears not be beneficial in determining severity of disease.

3.4.6 OCT to classify DMO

OCT has been used to propose a classification for DMO.[112] Their classification subdivided retinal thickening in DMO according to presence or absence of cystoid spaces and presence of subretinal fluid. However OCT findings had not been correlated to the current methods of assessing severity of diabetic maculopathy, namely FA, BCVA or slit lamp biomicroscopy. Thus this classification has not been universally accepted.

3.4.7 OCT in my study

OCT has become an important adjunct in the assessment and management of diabetic maculopathy. In my study I will perform OCT and use the CSFT to assess central macular function. I will correlate CSFT to both severity of disease and to the assessments of retinal function that I use in my study (mfERG, MP, OP and CS). In Chapter 5 I describe the technique I have developed to compare between OCT, mfERG and MP.

3.5 Diabetic Retinopathy Clinical Research Network

The DRCR.net is a collaboration of US institutes that lead multicentre research into diagnostic and therapeutic strategies for diabetic eye disease, including diabetic maculopathy.[113] It is funded by the National Eye Institute and the National Institute of Diabetes and Digestive and Kidney disease, a part of the National Institutes of Health (a branch of the US government that funds medical research). It is comprised of community-based and university-based practices. As of January 2014, a total of 179 sites and 786 investigators have joined the DRCR.net.

As part of the DRCR.net, several studies have been completed or currently being undertaken. Each study is referred to as Protocol A, B, C etc. Details of each protocol are available on its website (www.drcr.net). I will refer to specific protocols in the following paragraphs.

One study (Protocol B) prospectively compared treatment of CSMO with either focal or grid laser (n=330) to two doses of intravitreal triamcinolone (IVTA): 1mg (n=256) and 4mg (n=254).[114] Subjects were followed at 4 monthly intervals and BCVA and

OCT were recorded. Subjects were retreated if there were signs of inadequate response or worsening. Short term results showed the 4mg IVTA to be superior. At 24 months VA was better in the laser group as compared to the 1mg IVTA (p=0.02) and 4mg IVTA (p=0.002) groups. However VA only improved by 1 letter in the laser group. In addition use of IVTA was associated with an increased risk of developing elevated intraocular pressures and cataract surgery.

A pivotal DRCR.net study in the management of DMO was Protocol I which compared single and combination therapies of intravitreal anti-VEGF injections (ranibizumab), intravitreal triamcinolone injections and macular laser. In this prospective study of 691 patients (854 eyes), the authors compared four groups: i) sham injection + prompt laser (n=293), ii) ranibizumab + prompt laser (n=187), iii) ranibizumab + deferred laser for ≥24 weeks, and iv) triamcinolone + prompt laser.[115] Subjects were followed for a minimum of 12 months. Subjects who received ranibizumab, regardless of whether laser was prompt or deferred, had a significantly greater improvement in VA and reduction in OCT CSFT as compared to subjects with laser alone ($p<0.05$) at both 12 months and 24 months. The authors concluded that intravitreal ranibizumab should be considered for the treatment of DMO.

In Protocol A, the authors compared two methods of laser photocoagulation for diabetic maculopathy.[116] An OCT based system was developed to identify characteristics of DMO that would explain variations in VA and predict outcome after laser treatment. A total of 323 eyes were included. A database of 97 eyes of patients with diabetes but no maculopathy were used to determine normal mean values for each of the nine OCT subfields described above. A subfield was determined thickened if the thickness value was ≥3 standard deviations above the normal mean value. Associations between number of thickened subfields and a variety of outcome variables, including BCVA, CSFT and total macular volume were explored for both cross-sectional and longitudinal analysis. Better baseline VA was associated with fewer thickened subfields (r=0.38). However change in visual acuity showed no association with baseline number of thickened subfields at either 3.5

months (r=0.08) or at 12 months (r<0.01) after laser photocoagulation. Thus retinal thickness on OCT and VA were only moderately correlated.

These pivotal studies in the DRCR.net collaboration have advanced the management of diabetic maculopathy and the role of OCT in its analysis. Due to the impact of laser and intraocular treatment on changes in both VA and OCT thickness, I will exclude any subjects who have undergone treatment for diabetic maculopathy to ensure accurate comparisons between my groups. I will also compare retinal thickness to central macular function to determine correlation between OCT and mfERG, MP, OP and CS.

3.6 Systemic control

3.6.1 Steno-2 study

In this landmark prospective, randomised, parallel trial, Gæde et al (2003) compared two treatment regimes in the development of macro- and microvascular complications in subjects with type 2 DM.[117] Eighty subjects were treated with conventional therapy (as per the recommendations of the Danish Medical Association) and 80 subjects received intensive therapy (as per study protocol). Intensive therapy comprised a specific dietary regimen, a protocol for prescribing antihypertensive agents and oral hypoglycaemic agents. There was a mean follow up of 7.8 years with follow up every three months. Subjects in the intensive therapy group were significantly less likely to develop retinopathy (p=0.02) and blindness (level of VA was not reported) (p=0.03) as compared to the conventional group. The relative risk of development or progression of retinopathy was 0.42 (CI 0.21-0.86) in the intensive therapy group. The authors concluded that long term, targeted, intensive therapy reduced the risk of both cardiovascular and microvascular complications by about 50% in subjects with type 2 DM and microalbuminuria. However it should be noted the low numbers in each group with a 20% drop out rate by the end of follow up.[118]

3.6.2 Cholesterol

One feature of diabetic maculopathy is the presence of exudates, often accompanied by the presence of retinal thickening.[119] Exudates are formed as a

result of lipoproteins “leaking” from damaged retinal vessels into the extracellular space.

In the ETDRS 2709 patients with diabetes had baseline serum cholesterol levels available.[100] Fundus photographs were graded by the reading centre staff and the extent of hard exudates determined as per the criteria in Table 3.2.

Table 3.2: Grading of presence of hard exudate from colour photographs in the ETDRS (Std = standard)[119]

Severity Level	Description
None	No hard exudate present
Questionable	Questionable hard exudate present
Definite	Definite hard exudate but < Std photograph 3
Obvious	Hard exudate ≥ Std photograph 3 but < Std photograph 5
Moderate	Hard exudate ≥ Std photograph 5 but < Std photograph 4
Severe	Hard exudate ≥ Std photograph 4
Cannot grade	Unable to grade image

Of the 2709 patients, ⅔ were aged over 40 and 57% had a duration of DM ≥15 years. A multivariate logistic regression analysis was performed, adjusting for known confounders such as age, HbA1c, baseline retinopathy severity and extent of retinal thickening. Other confounders such as duration of diabetes or type of diabetes were not found to be statistically significantly associated with presence of hard exudate, with statistical significance set at $p \leq 0.01$. Analysis was performed on photographs graded as obvious or higher.

The presence of hard exudates was also associated with worsening visual acuity ($p=0.002$) even when adjusting for retinal thickness.[119] Increasing severity of hard exudates was associated with an increased risk of developing moderate visual loss (MVL, a decrease of ≥3 lines on the visual acuity chart); at 5 years ~45% of patients

with a grading of hard exudate of 'severe' at baseline developed MVL as compared to 15% of individuals with no hard exudates at baseline (odds ratio [OR], 2.27; confidence interval [CI] not specified; p<0.001). Even individuals with a grading of 'obvious' were 46% more likely to develop MVL (OR 1.46; CI not specified).

Patients with a cholesterol level of ≥6.21 mmol/l were twice as likely to have hard exudates as compared to those with a level less than 5.17 mmol/l (odds ratio [OR], 2.00; 99% confidence interval [CI], 1.35-2.95). In addition, Cox proportional hazards analysis of those in whom laser was deferred revealed that elevated levels of cholesterol were associated with the development of hard exudates 50% faster than those with levels below 5.17 mmol/l (OR, 1.54; 99% CI 1.17-2.02). Individuals with elevated serum cholesterol (>6.21 mmol/l) were 50% more likely to develop MVL as compared to those with level <6.21 mmol/l (OR 1.5; 99% CI 1.1-2.1).

However we do not know the number of patients in each group and whether they were on any lipid lowering agents, though only 15 of the 3711 patients enrolled into ETDRS were known to be on a lipid lowering agent. Also we do not know if there were any changes in serum cholesterol level over time as only baseline cholesterol levels were used for analysis. The authors have not explained why levels of 5.17 and 6.21 mmol/l were chosen for analysis, suggesting an arbitrary selection in post hoc analysis. Though the authors mention that eyes treated with laser at baseline were less likely to develop obvious hard exudates, there is no data provided to confirm statistical significance.

These results indicate that serum cholesterol, and retinal exudates, are a risk factor for developing visual loss and hence should part of the investigative process. Reduction of serum cholesterol forms an important part of the management of patients with DM; although it is often overlooked.

In a retrospective study of subjects with exudates in the presence of CSMO, mean serum cholesterol was assessed over a six month period.[120] Cholesterol levels were significantly elevated (p<0.02) in subjects with progressing exudates compared to subjects in whom the exudates had regressed. The former group also

demonstrated a significant decrease in BCVA ($p<0.01$). The authors concluded that strict lipid lowering therapy may result in better anatomical and visual outcomes.

An uncontrolled case series of patients with diabetic maculopathy and elevated lipid profile were treated with oral atorvastatin.[121] Subjects underwent clinical examination and FA at baseline and 3, 6 and 12 months. 18 eyes were included. At 12 months, subjects had a significant decrease in total cholesterol ($p<0.05$). In addition exudates and fluorescein leakage had regressed. However the authors have not reported any changes in other systemic risk factors such as blood pressure.

In the ACCORD Eye study the effect of intensive control of systemic risk factors was investigated in the development or progression of DR. Subjects were enrolled to intensive glycaemic control, intensive BP control or receiving simvastatin with either fenofibrate or placebo.[122] Intensive glycaemic control was associated with a 33% reduction in relative risk of progression of DR but there was no significant reduction in MVL. Intensive BP control offered no significant benefit in reduction of DR progression or MVL. Subjects in the fenofibrate group showed a significantly lower triglyceride level ($p<0.001$) but not HDL or LDL cholesterol level. These subjects also demonstrated a significantly decreased rate of progression (CI, 0.42-0.87; $p=0.006$) but no significant difference in MVL ($p=0.57$).

The FIELD study was a 4 year, multinational study that compared fenofibrate ($n=4895$) to placebo ($n=4900$) with outcomes being progression of retinopathy and requirement of laser for diabetic retinopathy in subjects with type 2 DM.[123] Subjects receiving fenofibrate had a significantly decreased requirement for first laser (HR 0.69, $p=0.002$). There was no significant difference in 2-step progression of DR between the two groups ($p=0.19$). There was no significant benefit of fenofibrate in reducing progression to DR in subjects with no DR at baseline ($p=0.87$); however, in subjects with pre-existing DR there was a significant reduction in progression with fenofibrate use ($p=0.004$). The authors also reported fewer instances of DMO in the fenofibrate group, though this was not significant ($p=0.09$). However, the subjects who underwent laser had no significant difference in serum cholesterol compared to those who did not receive laser; they did however have significantly higher

HbA1c, systolic BP, duration of DM, and rates of microvascular complications and were more likely to be on insulin compared to those who did not receive laser. The authors concluded that fenofibrate use, in addition to correction of other systemic risk factors, reduced need for laser treatment through reduction of inflammatory processes at the retinal capillary level rather than changes in serum lipid levels. One limitation of the study is that longitudinal values of systemic risk factors have not been reported. Therefore the need for laser or progression of retinopathy may be related to worsening systemic risk factors, rather than the effect of fenofibrate reducing risk. No correction for differences in systemic risk factors at baseline was made.

Serum cholesterol appears to play a significant role in the development of diabetic eye disease. In my study I will perform serum cholesterol levels at baseline and in subsequent follow-up visits. I will record lipid-lowering agent use at baseline. Where appropriate, corrections for differences in serum cholesterol between groups will applied to data analysis.

3.6.3 Glycated serum haemoglobin level (HbA1c)

Serum HbA1c is the gold standard for the assessment of control of DM and has more recently gained acceptance for the diagnosis of DM.[124] Association between serum HbA1c and the development of microvascular complications secondary to DM has been extensively studied.

The United Kingdom Prospective Diabetes Study (UKPDS) was a landmark, multicentre, randomised trial that assessed therapies for DM in subjects with type 2 DM. It received funding from the UK Medical Research Council, US National Institutes of Health, British Diabetic Association, along with several other local and commercial groups. In Report 35, the investigators longitudinally evaluated levels of glycaemia in the development of macro- and microvascular complications of DM.[125] Subjects were allocated to two groups: conventional control (n=1138) aimed for a fasting plasma glucose concentration <15.0 mmol/l and intensive control (n=2729) aimed for a concentration <6.0 mmol/l. The authors reported that increase in mean updated HbA1c was associated with an increased risk of development of

complications even after adjustment for various baseline variables. Though there was no specific analysis for development of DR or maculopathy, the authors reported that for every 1% reduction in HbA1c, there was a 37% decreased risk of developing microvascular complications and a 19% decreased risk of requiring cataract extraction. The authors concluded that any improvement in glycaemic control is likely to reduce risk of development of complications.

The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study was a parallel-group, randomised, multicentre trial that investigated the control of systemic factors in the development of diabetic microvascular complications.[126] In the core study subjects were randomised to intensive glycaemic control (HbA1c <6.0, n=5128) or standard glycaemic control (HbA1c 7.0-7.9, n=5123). In addition subjects were randomised to receive either intensive BP control or a lipid intervention depending on baseline levels of both. Intensive therapy offered no significant reduction in risk of developing microvascular or ocular complications. However there was an increase in total mortality in the intensive group and so the study was stopped before reaching completion. The authors therefore concluded that tight glycaemic control may neither be achievable nor desirable.

The Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) study investigated the effect of intensive glycaemic control on rates of macrovascular and macrovascular complication development.[127] A total of 11,140 subjects aged 55 years or over with type 2 DM were randomised to either standard or intensive glucose control. Though there was reduced frequency in the major macrovascular events (p=0.01), there was no significant effect on DR development (p=0.50) or visual deterioration.

The Veteran Affairs Diabetes Trial (VADT) assessed the effect of intensive glycaemic control on retinopathy.[128] There was no significant change in either worsening of retinopathy or development of CSMO, though there was a trend in favour of intensive control (p=0.07). However the study was limited through having a predominantly male cohort, restricted availability of newer drugs and being underpowered.

In a cross-sectional study, Zander et al (2000) studied association between diabetic maculopathy and various risk factors.[129] A total of 1796 patients with type 1 DM and 1563 with type 2 DM were recruited. Clinical examination and FA were used to grade DR and assessing maculopathy. Diabetic maculopathy was deemed present if any of the criteria for CSMO was met. Maculopathy was reported in 15% of subjects with type 1 DM and 28% of subjects with type 2 DM. HbA1c levels were not deemed to be significantly different between subjects with maculopathy and those without. However elevated serum creatinine ($p<0.01$) and serum cholesterol ($p<0.01$) were reported in subjects with type 1 DM and maculopathy. Other risk factors significantly associated with prevalence of maculopathy were increasing duration of DM in both type 1 and type 2 DM, elevated cholesterol levels in type 1 DM and elevated BP in type 2 DM. However the authors have not reported medication use; therefore prior systemic control of HbA1c cannot be determined.

A community based survey in Taiwan of patients with type 2 DM aimed to find a relationship between the number of MA and systemic risk factors.[130] In 527 patients, a single 45^{o} colour photograph centred at the macula was taken to determine presence and location of MA. Grading revealed 83.5% of patients had no DR, 6.3% demonstrated only MA and 10.2% demonstrated MA with other diabetic retinal lesions. Elevated HbA1c was associated with more MA ($r=0.38$, $p=0.03$). Every 10% increase in HbA1c was associated with an increase of 0.7 MA. Multiple logistic regression demonstrated duration of DM and HbA1c to be significantly associated with increasing level of retinopathy. Systolic and diastolic BP and cholesterol were found to be non-significant. As this study was cross-sectional it is probable that the retinopathy reflected previous systemic control. This may explain why cholesterol and blood pressure were found not to be clinically significant. Only MA present within the macula were considered; so patients with MA in the peripheral retina were considered to be free of DR. Those subjects excluded were found to be significantly older than the group studied, introducing possible selection bias. Only patients with type 2 DM were included; so results may not be applicable in type 1 DM.

One important consideration is the improvement in systemic control over the years. In the ETDRS, 40% of patients had a HbA1c of ≥10%.[67] In the DRCR.net only 17% of subjects with an HbA1c of ≥10%.[131] Therefore more recent studies are likely to have a patient population comparable to mine.

HbA1c, unlike cholesterol, has not been shown to be associated with diabetic maculopathy. Nevertheless, it remains an important marker of DM control and is routinely assessed in clinical practice and research in diabetic eye disease. I will record HbA1c and perform inter-group comparisons. Where appropriate I will correct for HbA1c for such comparisons.

3.6.4 Blood pressure

In the UKPDS Reports number 36, 38 and 60, the relation between systolic BP and risk of developing complications in type 2 DM was assessed.[132]

In Report 36, the incidence of developing complications was assessed in 4801 patients, and 3642 patients underwent evaluation for potential confounders.[133] Ophthalmic end points were retinopathy requiring laser treatment and vitreous haemorrhage. The authors reported a twofold increased risk of developing any complication over a systolic BP range from 114 mmHg to 168 mmHg. Incidence of microvascular complications increased from <10 per 1000 years in the group with systolic BP <120 mmHg to 25 per 1000 years in the group with systolic BP >160 mmHg. There was no threshold limit for any complications. The authors also reported that for every 10 mmHg decrease in systolic BP, the risk of developing microvascular complications decreased by 13% ($p<0.00001$).

In report 38, the authors compared subjects who received intensive BP control (<150/85 mmHg, n=758) and those who received less intensive control (<180/105 mmHg, n=390).[134] Baseline characteristics were similar between the two groups. Median follow up was 8.4 years. Mean BP at end of study was 144/82 mmHg and 154/87 mmHg in the intensive and less intensive groups, respectively. The author reported 37% reduction in risk of microvascular disease (mainly retinal photocoagulation) and 47% reduction in decrease in VA by ≥3 lines on ETDRS chart

in the intensive control group. Sub-analysis revealed no significant difference in risk of vitreous haemorrhage, most likely due to patients receiving treatment for PDR.

In report 69, 1148 patients were assessed for development of retinopathy and maculopathy over 7.5 years.[132] Subjects in the intensive BP control group developed significantly fewer exudates at 7.5 years (RR 0.53; $p<0.001$) and were less likely to require photocoagulation for maculopathy (RR 0.58; $p=0.02$). However the authors did not comment on rates of development of CSMO or ischaemic maculopathy. There was a small reduction in risk of developing MVL (Snellen VA 6/60 or worse) of 3.1% in the intensive control group compared to 4.1% in the less intensive control group (RR 0.76, $p=0.46$). The authors concluded that high BP was related to an increased risk of developing diabetic eye disease and tight BP control reduced risk of diabetic eye disease.

In the ACCORD Eye study, subjects were randomised to intensive vs standard blood pressure control.[122] At 4 years there was no significant difference in progression of retinopathy ($p>0.2$) or rates of MVL ($p>0.1$) between the two groups. However no results were published with respect to formation of diabetic maculopathy.

Elevated BP is associated with an increased risk of developing diabetic retinopathy. In my study I will record BP for inter-group comparisons and, where appropriate, adjust comparisons accordingly.

3.7 Functional assessment of diabetic maculopathy

As described above the diagnosis of diabetic maculopathy is currently based on clinically visible changes combined with OCT findings to determine management of the maculopathy. More recently studies have shown that neural changes within the retina precede vascular changes. These neural retinal changes may be detected by assessing retinal responses to stimulation by light. These responses can be detected both objectively by electrodiagnostic testing (multifocal electroretinography, oscillatory potentials) and subjectively by microperimetry. I intend to use both methods to assess retinal function in different stages of diabetic maculopathy.

3.7.1 Electroretinography

In the middle of the nineteenth century, animal experiments identified that eyes carried an electrical potential.[135] Further experiments identified the retina to be the source of the electrical potential and that it responded to light. In 1877 Dewar performed *in vivo* experiments leading to the first successful recording of the human electroretinogram (ERG).[135] The following 50 years saw an improvement in the recording equipment and clearer delineation of the electrical potential obtained in the presence of flashing lights. Extensive investigation by Granit in the fourth and fifth decades of the 20th century resulted in the identification of the various components of the ERG that are still used today.[135]

3.7.2 Components of an ERG

The wave pattern of an ERG represents the various structures of the neural retina and is illustrated in Figure 3.6. The initial negative deflection, the a-wave, represents photoreceptor activity and is the first response seen after retinal stimulation by a flash of light. The following positive deflection is termed the b-wave and represents fluxes of potassium ions in Müller cells, though is highly dependent upon photoreceptor and bipolar cell function.[23]

3.7.3 Advent of the multifocal electroretinogram

The standard ERG evaluates the function of the retina as a whole, full field ERG.[135] Though this has benefits for assessing many retinal disorders, it does not provide information on localised disease. This led to the development of the multifocal electroretinogram (mfERG).

The mfERG was demonstrated by Erich Sutter in 1991 and allows for simultaneous measurements of multiple retinal responses at different locations.[136,137] It produces a topographical map of retinal function covering the central 40-50^{o} of the retina, providing information on macular function.

There are several commercially available systems for recording mfERG: the VERIS system (Electro-Diagnostic Imaging, San Mateo, CA), the RETIscan system (Roland Consult, Wiesbaden, Germany), and the Metrovision system (Metrovision, Pérenchies, France). Our unit utilises the RETIscan system. Each system has its own

set of parameters which influence the data collected. The International Society for Clinical Electrophysiology of Vision (ISCEV) has produced guidelines to aid clinical scientists and researchers with standardisation of test parameters and interpretation.[136]

The standard mfERG predominantly measures cone function.[136] There are 2 responses produced by the mfERG that are most commonly analysed: the first order kernel and the second order kernel.

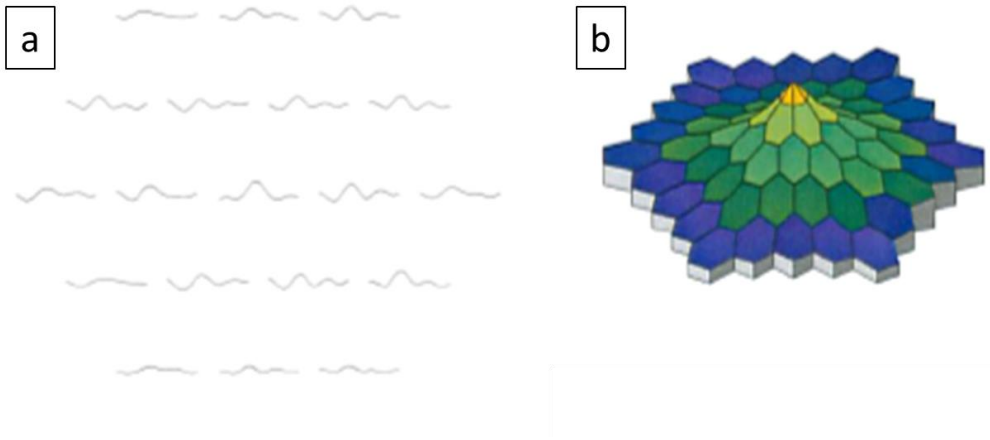
The first order kernel represents the largest response and is a biphasic wave. It is the one that is most commonly presented as clinical data. This kernel is obtained by adding all the results generated by a bright flash and then subtracting away all the results generated by a dark flash. Experiments suggest that the first order kernel response represents cells in the outer retina, i.e. the photoreceptors and the on and off bipolar cells.[136]

The second order kernel is a much smaller response and suffers from a poorer signal-to-noise ratio. Though this component was meant to represent ganglion cell activity, it appears to be more non-specific and is instead used to assess how the mfERG response is affected by the preceding flash.[136]

3.7.4 Analysis of mfERG responses

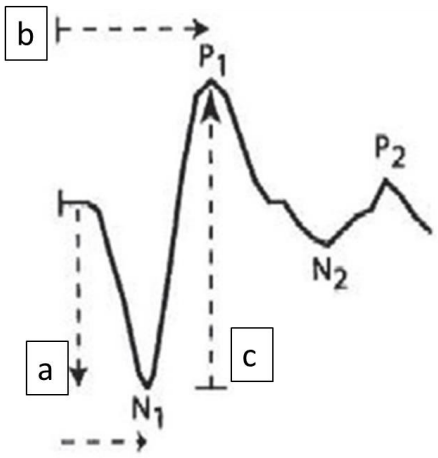
The responses generated by the mfERG may be analysed by several methods. Each discrete response may be analysed individually. More commonly, the responses are grouped and averaged for each ring structure. In my study, I utilise a 19 hexagonal array centred on the fovea (Figure 3.5a). Each hexagon, when stimulated, produces an individual waveform which can be studied. A three-dimensional topographical map is produced so the clinician can visualise any defects in retinal function (Figure 3.5b).

Figure 3.5: Output of a 19 hexagon mfERG array: a) waveform generated for each hexagon; b) 3D topographical representation of amplitudes relative to the standard normal



The main parameters recorded from the waveform are amplitude and implicit times (Figure 3.6). In my study amplitude recorded is from the first order kernel and is the height of the wave from the N1 trough through to the P1 peak. Implicit time is measured from the onset of stimulus to the peak of the waveform. Amplitude represents the strength of the response while implicit time represents speed of the response from stimulus.

Figure 3.6: Schematic representation of the ERG response. Dashed lines represent stimulus (a); time from stimulus to 1st peak (implicit time, b); height of the response (amplitude, c)



Patients are light adapted for 15 minutes prior to commencement of data acquisition with the lights left on for constant light adaptation. This is because alterations of extent of retinal bleaching will affect the results obtained.[136] Monocular recording is carried out as it facilitates better fixation monitoring.[136]

3.7.5 Factors affecting mfERG results.

Patients are maximally dilated prior to testing. This is to maximise retinal illumination as changes in pupil size results in significant alterations of the amplitude and latency recorded.[136] Alterations in room lighting have been shown to affect retinal bleaching and hence results obtained. Thus it is essential that patients are light adapted prior to recording.

Increasing age is associated with a decrease in amplitude and increase in implicit time.[137] Studies have reported a 5 to 10.5% reduction per decade in P1 amplitude and a 1 to 1.2% increase in P1 implicit time per decade.[138,139] This effect seems to be more pronounced in the central retina compared to the peripheral retina. The causes of these age-related changes are a combination of optical factors and decreased neural retina function, such as loss of photoreceptors and inefficient synaptic transmission.[136,138]

The presence of mild or moderate cataract leads to a reduction in P1 amplitude as compared to patients with very mild cataracts.[140] This effect appears to be due to light scattering. Patients who underwent cataract demonstrated an improvement in mfERG responses.[136,141] There did not, however, appear to be significant change in implicit times.[142] Interestingly, light scattering conversely increases peripheral mfERG responses.[117]

Refractive errors and axial length also appear to influence mfERG responses. Amplitudes were reduced and implicit times delayed as refractive errors increased.[143] Increased axial length also results in reduced amplitudes. One postulated cause is the loss of cone function associated with high myopia. Chen et al[144] demonstrated prolonged implicit times in myopic patients. They also

demonstrated that refractive error appeared to have a greater effect than axial length.

Optical defocus has been shown to reduce P1 amplitude of the mfERG response by 12 percent with blurring of both +1.00D and +3.00D.[145] It is postulated that the area stimulated was larger than the hexagon resulting in reduced contrast of the image. The effect appeared to be greater in the central macula as compared to the peripheral macula. However there did not appear to be any influence on implicit time. However this study was performed on the VERIS system with a 103 hexagonal pattern.

A study of eight patients found no significant difference in amplitude or latency in the central 4^{o} but significant difference in the peripheral 6 to 25^{o} due to refractive blur using the Retiscan system.[146] They recommend refraction should be corrected prior to testing. They also demonstrated that refractive errors of up to 6 dioptres did not influence amplitude or implicit time.

Therefore mfERG appears to be a useful test. There are several parameters which could influence the results and make them difficult to interpret. Nevertheless, mfERG provides an objective method of assessing neuronal function in the macula and should be useful in assessing diabetic maculopathy.

3.7.6 Application of mfERG in diabetic retinopathy

MfERG has been used in several studies to assess retinal function in the presence of DM. Most of these studies have found implicit time to be more sensitive than changes in amplitude in detecting retinal dysfunction.[136]

Harrison et al (2011) reported on a prospective study of 78 eyes of 48 patients with DM.[147] The authors aimed to predict development of DR in patients with no retinopathy at baseline using mfERG implicit time (IT) and were followed for between 1 to 6 years (mean 3 years). All patients also underwent HbA1c measurement and dilated fundus examination with colour photos. The mfERGs were recorded using the VERIS 4.3 system with a 103 hexagon stimulus array and a frame rate of 75Hz subtending 45 degrees of the retina. The mfERG IT of 50 control

subjects was used for comparison. A Z-score was assigned to each implicit time of diabetics using the mean and standard deviation of controls. The authors reported a 20% (OR 1.16, 1.02-1.33) increase in risk of developing retinopathy with a one unit Z-score increase (i.e. delay) in mfERG IT. Duration of DM was reported as a risk factor too, with risk of developing retinopathy increasing by 7% (OR 1.07, 1.00-1.15) with every increasing year of duration. Patients with type 1 DM appeared to be at greater risk of developing retinopathy with comparatively small delays in implicit time.

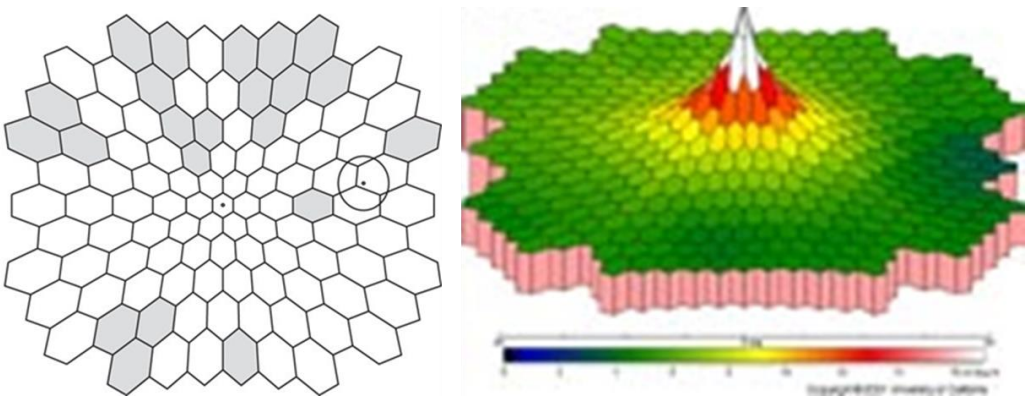
A study of 37 eyes in 27 patients looked at the presence of exudate and its effect on mfERG recordings of amplitude and IT.[148] The authors reported a statistically significant prolongation of IT in areas of exudates compared to those without exudate. Amplitude was only reduced in the parafoveal and extrafoveal regions. The authors concluded that IT was prolonged as retinal capillaries are affected in DR and are responsible for supplying the inner nuclear layer of the retina, the same region as the bipolar cells. Limitations of the study include small numbers of patients (15 eyes with exudates and 16 eyes without exudates), lack of information on severity of DR or maculopathy, and no assessment of macular ischaemia. Also, no explanation was given for how overlapping hexagons and the OCT were correlated.

In a prospective cross-sectional study of 26 eyes of patients with DM with background DR,[149] mfERG was performed on the VERIS system and used to evaluate macular function and correlate with macular thickness and medical parameters. Values recorded for mfERG included amplitude and IT for the central subfield, middle ring and outer ring corresponding to the ETDRS grid. Sixteen of the 26 eyes had macular oedema. Increase in central macular thickness was associated with a reduction in amplitude ($r= -0.541$, $p= 0.004$) and a prolongation of IT ($r= 0.548$, $p= 0.004$) in the central area of the mfERG. BCVA was positively correlated with amplitude in the central area of the mfERG ($r= 0.630$, $p= 0.001$), but there was no correlation with IT. There was no correlation between mfERG and size of FAZ, though mean area of FAZ was 600 ± 24µm. No details were provided on architecture of the FAZ or the perifoveal capillaries. Increasing age was significantly associated with a reduction in central amplitude ($r= -0.514$, $p= 0.029$) but not

prolongation of IT. Increasing duration of DM was paradoxically associated with higher amplitudes (r=0.496, p=0.043) and shorter IT (r= -0.573, p= 0.016). No correlation was found between HbA1c and systolic BP. Subgroup analysis revealed a macular thickness greater than 300µm was associated with a significantly lower mfERG amplitudes (15.7±10.0 vs 29.3±10.0 nV/deg^2; p=0.002), longer IT (32.5±3.6 vs 29.1±4.5 ms; p=0.01) and lower VA (70 vs 87 letters (p=0.001). However no multivariate analysis was performed to adjust for factors such as age. Though retinopathy was graded, the authors did not state the severity of macular oedema and no comparison was made to healthy controls.

In my study I use a 19 hexagonal mfERG array. However several studies have used 103 hexagons (Figure 3.7).[147,150-153] In these studies, rather than analysing individual hexagons, retinal zones are created where the amplitudes and IT of one hexagon and its surrounding hexagons are analysed together as one zone. Therefore 35 zones are created. The difficulty with using so many hexagons is the time required to record responses, the poor signal to noise ratio and the post-procedural analysis required to analyse the individual retinal zones. The long duration of recording can be uncomfortable for patients. In addition, smaller stimuli have been shown to be more susceptible to fluctuations in fixation.[154] Subsequently, a 61 pattern hexagon was developed.[155] Though the duration of recording was reduced, the poor signal to noise ratio and the need for post-procedural analysis still remained. Therefore the 19 hexagonal array is used in our unit.[137]

Figure 3.7: Schematic of the 103 hexagonal array with a corresponding 3D topographical representation of output



In my study I will assess central macular function using mfERG in varying severity of diabetic maculopathy. I will assess both amplitude and IT and correlate them to retinal thickness. I will also evaluate change in amplitude and IT over 6 and 12 months to determine whether change in function correlates to severity of disease.

3.7.7 Oscillatory potentials in diabetic retinopathy

Along the ascending limb of the b-wave of the ERG are small waves of increased frequency. These are known as oscillatory potentials (OPs) and are thought to originate from the inner retinal layers, especially the amacrine cells.[156,157] ERG studies have shown these cells to be preferentially affected in diabetic retinopathy. As the retinal circulation is located within the inner retina, and DM is associated with disruption of this circulation, it is not surprising that OPs may be affected.

In a retrospective study with a 6-8 year follow up, subjects with NPDR and abnormal OP amplitudes were more likely to progress to PDR than subjects with normal OP amplitudes (53% vs 4%).[158] However information regarding severity of NPDR was not provided and follow up varied between subjects. No information was provided on level of maculopathy.

As mentioned above, current mfERG stimuli assess function of the bipolar cells with only minor contribution from the amacrine cell. Thus assessing retinal dysfunction using OPs is likely to yield greater information. There have been some studies that demonstrate a delay in OP IT in patients with DM without DR[155] in addition to a reduction in amplitude in patients with pre-proliferative DR.[159] However, these studies averaged large areas of the retina rather than assessing local responses.

Reduction in OP amplitude has been associated with DR severity.[156] In a longitudinal study of 80 insulin-dependent diabetics with no clinical signs of DR, 35% developed DR at final follow up.[160] OP amplitude had decreased by 46% in this group, compared to a 25% drop in those who did not develop DR. This difference was statistically significant ($p<0.001$). In addition of those who developed DR, 61% of eyes demonstrated a decrease in OP amplitude prior to development of DR. The authors concluded that subnormal OP amplitude may identify those at risk of

developing DR. However the study did not report on development of DMO or CSMO. The study only included patients below the age of 40.

In a prospective study of 24 eyes of 12 patients with type 1 DM and no DR, and 26 eyes of 14 healthy patients, the authors performed multifocal OPs to assess changes in implicit times and amplitude.[155] Recordings were performed on the VERIS system using a 61 hexagonal pattern. For analysis one eye of each patient was selected at random and the authors comment on two peaks generated by the recording. The authors concluded that there was a significant delay in IT at fovea in subjects with DM compared to healthy controls (22.22±0.95ms vs 22.95±0.92ms) for the first peak but not for the second peak. However there was no significant difference in amplitude. The authors concluded that multifocal OPs demonstrated a dysfunction of the inner retina with rods more likely to be affected.

A study of 32 eyes of patients with DM (16 with no DR and 16 with pre-proliferative DR) looked at different components of the OP waveform.[157] The authors identified that individuals without DM had a statistically significant greater signal to noise ratio (SNR) as compared to patients with DM, suggesting greater retinal function. No further analysis was carried out on comparing diabetics with controls. They also identified decreased SNR with increasing severity of DR, though statistical significance was not reported. This study identified a greater abnormality in the 2nd order kernel of the OP waveform as compared to the 1st order kernel in patients with exudates or macular oedema, though statistical significance was not reported. In addition there were only small numbers in the groups (20 with hard exudates and 10 with oedema). The authors do not describe the severity of these lesions in relation to their size and location from the fovea and no analysis was performed in relation to maculopathy status.

The role of OPs in detecting progression was studied by Bresnick et al (1984).[161] Eighty-five eyes of subjects with DM who took part in the ETDRS underwent ERG at baseline at various stimulus intensities. Level of retinopathy was recorded and the end point was progression to PDR with high risk characteristics (HRC). A cut-off point of 75 µV for OP sum amplitude was selected as 95% of the non-diabetic

population studies by this group achieved a value greater than this. There were 25 eyes in the abnormal group and 60 eyes in the normal group with a median follow up of 23.5 months and 29.0 months respectively. Subjects in the abnormal group were more likely to progress to PDR-HRC ($p<0.0001$). Rates of progression were 28% in the abnormal group compared to 0% in the normal group at 1 year, 34% and 7% respectively at 2 years, and 62% and 13% respectively at 3 years. When adjusted for retinopathy level hazard ratio for progression was 10.0 ($p<0.001$) for those in the abnormal group compared to those in the normal group. Sub-analysis of subjects with PDR at baseline revealed that those within the abnormal group were more likely to progress than those with a normal amplitude (hazard ratio 21.7, $p<0.001$). This study did not report on maculopathy levels. In addition no information is provided on the demographic values of the subjects included within the study.

OPs were compared between patients with different severity of retinopathy (n=174) and healthy controls (n=54).[156] The authors looked at different stimulus intensities and found a statistically significant reduction in mean amplitudes across all patients with DM compared to controls (at stimulus intensity of 0.5, amplitude was 112.8µV vs 175.0µV, $p<0.001$). The data was further subdivided into types of retinopathy, but maculopathy status was not reported. The authors reported no significant difference between healthy controls and subjects with no DR and mild to moderate NPDR. Regression analysis identified that vascular leakage on FA was a significant predictor of OP amplitude compared to capillary non-perfusion. However non-perfusion was not defined in terms of location, severity, and area of retinal involvement. Also data was not provided on visual acuity.

Electrodiagnostic tests, such as mfERG and OP, appear to show reduced macular function in the presence of DM, with increasing severity of disease. MfERG appears to be more studied than OP and, as such, mfERG is being used in a multicentre study (European Consortium for the Early Treatment of Diabetic Retinopathy, EUROCONDOR) as a primary outcome (*Simon Harding, personal communication*). However, most studies have focused on analysis of diabetic retinopathy with limited information on diabetic maculopathy. Both amplitude and IT in mfERG and OP

appear to be affected. However, variability in stimuli and recording systems makes comparisons between studies difficult. In my study I will be performing mfERG and OP on the same subjects with the same recording system. This should make comparison between mfERG and OP more reliable.

3.7.8 Microperimetry

Microperimetry (MP) is an investigative tool that measures foci of retinal sensitivity and is an established technique for assessing macular function, at least in research active centres. Similar to fundus perimetry as used in the assessment of glaucoma, this subjective tool allows for the localisation of any reduced macular function. In my study I have used the Nidek MP1 microperimeter (Nidek Technologies, Padova, Italy) as it incorporates an eye tracker, allows for automated examination of the same retinal loci at follow-up visits, and can be co-registered to a colour image and therefore assessed in conjunction with pathology.[162]

Microperimetry has been used to study various macular disorders such as geographic atrophy[163] and subfoveal choroidal neovascularisation[164] as well as monitoring response to laser treatment for diabetic macular oedema.[165]

In a study of 50 eyes with various macular diseases, Chen et al (2009) studied the test-retest variability of the MP1.[162] Subjects underwent a 68 loci grid covering the central 20^{o}, performing the test twice on the same day with a break in between testing. A 95% co-efficient of repeatability was used to demonstrate variation between the tests. Results demonstrated that analysis of individual points (point wise sensitivity) had a coefficient of repeatability of 4.94 dB (SD 0.96), with 50% of points varying by 1 to 6 dB. The mean macular sensitivity (i.e. combining all the points) had a coefficient of repeatability of 1.81. The authors concluded that the mean macular sensitivity would not give sufficient topographic information and point wise sensitivity demonstrates too much variability to be clinically useful. However, if the central 16 points covering the central 5^{o} were analysed (central macular sensitivity) then the coefficient of repeatability improved to 2.04 dB. Thus the authors recommended combining clusters of loci to assess a region.

A cross-sectional study of 39 subjects with DM but no DR and 39 controls reported a significant reduction in mean retinal sensitivity covering the central 20^{o} (15.74dB vs 17.70dB, p=0.003).[31] The MP grid comprised of 33 stimuli and a stimulus projection of 120 ms was used. Sensitivity was significantly reduced in patients below the age of 50, but not above this age. There was no signification association with duration of DM or HbA1c. This study concluded that functional deficits appeared prior to vascular changes. However it excluded subjects below the age of 40 and analysis of sensitivity was not adjusted for age. The authors looked at sensitivity of the whole macula rather than just the central macula.

In another study of 152 eyes of 99 healthy subjects, Rohrschneider et al (2008)[166] reported a mean reduction of 0.275 dB per decade, with a starting sensitivity of 16.6 dB at age 10. The study used the MP1 instrument with a pattern comprised of 53 loci within a 20^{o} field centred on the fovea, with a stimuli projection of 120 ms. However, this was a cross-sectional study and results extrapolated from the whole group.

Vujosevic et al (2006)[167] prospectively compared microperimetric findings and OCT based retinal thickness in 32 patients with DM. A standard OCT scan and a microperimetric 45 stimuli radial grid covering the central 12^{o} of the macula were used. Stimuli projection of 200 ms was used for this study. BCVA was calculated using an ETDRS grid with the letter score converted to logMAR. Patients were divided into three groups: no macular oedema (n=16 eyes), macular oedema but not CSMO (n=30 eyes) and CSMO (n=15 eyes). Macular thickness increased significantly from the no oedema group to the CSMO group ($p<0.0001$). There was no statistical difference in macular sensitivity in the central field between the no oedema group and the not CSMO group, but there was a significant reduction in mean sensitivity in the CSMO group compared to the other groups ($p<0.05$). There was a significant correlation between retinal thickness and macular sensitivity in the CSMO group (r=-0.48; $p<0.0001$) but not within the other groups. Though there was a statistically significant correlation between VA and retinal sensitivity in the central five fields of the OCT grid in the not CSMO group, interestingly the same was not true for the CSMO group. The authors concluded that sensitivity decreased with

increasing severity of macular oedema. However the authors have not assessed for presence of macular ischaemia or reported on retinopathy severity. No comparison was made to healthy controls.

In a study of 20 eyes of 15 patients with type 2 DM and DMO, Soliman et al (2010) studied microperimetric findings in the presence of specific retinal lesions.[168] Patients were tested with a 73 stimuli pattern covering the central 10^{o} and a projection time of 200 ms, CF and FA to assess retinal structure and function. Sixteen eyes had CSMO and 4 eyes had a lesser degree of macular oedema. Macular sensitivity was negatively correlated to foveal exudates (r=-0.56, p=0.01), petalloid appearance of foveal leakage (r=-0.50, p=0.02), and honeycomb appearance of foveal leakage (r=-0.80, <0.0001). There were non-significant negative correlations between macular sensitivity and presence of retinal haemorrhage, diffuse leakage, focal leakage and ischaemic areas. The authors did not comment on differences in macular sensitivity between CSMO and non-CSMO macular oedema, or on macular sensitivity in presence of enlarged FAZ which will be part of my study. Foveal sensitivity was positively correlated to VA though this was of borderline significance (r=0.44, p=0.051).

One advantage of MP is that it allows for automated determination of stability of fixation. The built in tracker records foveal fixation and records percentage of points seen within central 2^{o} and central 4^{o} of fixation. If >75% of points are located within central 2^{o} then fixation is deemed stable, <75% within 2^{o} but >75% within 4^{o} fixation is relatively unstable, and <75% within 4^{o} is unstable. In a study of 84 eyes with CSMO, 59.5% of eyes showed unstable fixation.[159] However mean BCVA was 6/24. In another study of 178 eyes with DMO, 25.7% of eyes were deemed relatively unstable or unstable.[170] Presence of exudates, rather than oedema, was deemed significant. Mean VA was better than the previous study at 6/15. However, 90% of subjects with VA worse than 6/60 had poor fixation stability.

In the studies on MP described above, there are considerable variations in the study parameters. The researcher, or clinician, can adjust the number of stimuli, the size of fixation target, the stimulus projection time and the size of the overall field

projected, amongst others. Although this allows for targeted assessment of specific conditions, it makes it difficult to compare results between studies. In my study, I have developed a 48 stimuli grid which covers the central 32^{o}, with a stimulus projection time of 200 ms. This grid pattern was developed so that it corresponded to the mfERG 19 hexagon array. I have described this in more detail in Chapter 5.

In my study I combine the central 5 points covering the central ring of the ETDRS grid and take a mean value to calculate mean foveal sensitivity. My aim is to look at foveal sensitivity and adjust the analysis for various variables such as age, duration of diabetes, etc. I will address these issues as well as analyse changes in retinal sensitivity longitudinally, which was a recommendation of this paper. I will analyse foveal sensitivity in relation to the greatest linear diameter of the foveal avascular zone.

3.8 Discussion

Current methods of diagnosing and analysing diabetic maculopathy are based upon studies performed over 30 years ago that highlighted specific pathological findings. However by this stage the patient is likely to have developed visual impairment which may be permanent. Recent studies have demonstrated that neural changes may be detectable in patients with DM prior to developing DR.

Assessment of neural function of the macula has been performed in DM, using mfERG and MP. Most studies have focused on DR with few studies assessing macular function in diabetic maculopathy. Overall, retinal function appears to decrease in the presence of severe diabetic eye disease with conflicting evidence in early disease. OP has previously been studied in DM but appears to have gone out of favour, with early studies suggesting OP may be beneficial in assessing diabetic eye disease.

With improvements in imaging and the availability of newer assessments of neural function, macular function can now be studied to improve our understanding of the retina in disease. However there appeared to be a lack of co-ordination between structural and functional assessments. With the advent of tracking systems that co-

register functional assessments to retinal structures, it is possible to analyse structural and functional changes in the retina simultaneously. Therefore I have developed a technique of multimodal analysis which I describe in Chapter 5.

Diabetic maculopathy, especially DMO, remains the leading cause of visual disturbance in subjects with DM. Therefore, the aim of my study is to assess central macular function in subjects with varying severities of diabetic maculopathy. Initially, I aim to determine level of function in these subjects with respect to their maculopathy and subsequently monitor how function alters over time. In Chapter 4 I describe the methods used in my study and introduce the outcomes I will be analysing.

Chapter 4 Methods and materials

In this chapter I describe the methods of patient screening and recruitment of subjects with diabetes mellitus (DM) and healthy controls, the patient characteristics for inclusion into the study, and the protocols for systemic examination, multifocal electroretinography (mfERG), oscillatory potential (OP), microperimetry (MP), colour photography (CF), optical coherence tomography (OCT) and fundus fluorescein angiography (FFA). I will also describe the study design, data collection and statistical methods used for which I follow STROBE guidelines.[171,172] In addition I will describe the terminology used in my study.

4.1 Definitions

Macula: area located within the temporal vascular arcades, centred on the fovea and measuring roughly 5500µm in diameter[173,174]

Fovea: central retinal depression measuring 1500µm in diameter[174]

Foveola: maximum depression at the macular centre measuring 350µm in diameter[174]

Foveal avascular zone: area within the centre of the fovea lacking capillaries and bordered by an interlinked vascular supply as seen on FFA; in health measures less than 1000µm maximally

Clinically significant macular oedema (CSMO): as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS),[66] it refers to the presence of at least one of the following features: retinal thickening, any part of which is within 500µm of the centre; exudates within 500µm of the centre of the fovea, with associated retinal thickening; retinal thickening measuring more than 1 disc area in size, any part of which is within 1 disc diameter of the centre of the fovea (described in detail in Chapter 3)

Early maculopathy: the presence of macular oedema and/or exudates within the macula but which does not meet the criteria for CSMO

Ischaemic maculopathy: increase in the greatest linear diameter (GLD) of the perfused perifoveal capillary arcade as seen on FA to ≥1000µm

4.2 Recruitment of subjects with diabetes mellitus

Pre-screening of subjects was commenced in September 2010. Patients were recruited predominantly from the diabetic retinopathy screening assessment clinic and a dedicated diabetic retinopathy clinic, both based in the Clinical Eye Research Centre (CERC) of St. Paul's Eye Unit, Royal Liverpool University Hospital (RLUH), UK. Other sources of referral included the general and specialist clinics of the St Paul's Eye Unit and the Liverpool Diabetic Eye Screening Programme, also based at the RLUH. The inclusion and exclusion criteria are shown in Table 4.1.

Table 4.1: Summary of the inclusion and exclusion criteria for the study

Inclusion Criteria	Exclusion Criteria
Patients diagnosed with type 1 or type 2 DM by general practitioner	Age < 18 years
Best corrected visual acuity greater than 35 ETDRS letters (equivalent to 6/60 on the Snellen chart)	Patient unable to fully understand the informed consent process or comply with the study investigations
	Any ocular disease that may affect the blood-retinal barrier (e.g. vascular occlusions)
	Previous macular laser to study eye
	Peripheral laser photocoagulation performed within the previous 6 months
	Any intraocular procedure performed within the previous 3 months
	Any media opacities, abnormalities in pupillary dilatation or patient posturing which would preclude good quality image acquisition

4.3 Recruitment of controls

Controls were recruited from patients attending the St. Paul's Eye Unit at Royal Liverpool University Hospital, members of staff based at the hospital and friends and relatives of members of staff.

The inclusion/exclusion criteria for controls were similar to subjects with DM, except that the controls did not have DM. The absence of DM was confirmed through the oral glucose tolerance test (OGTT), which was carried out as per local protocol and comprised the following steps:

1) Collection of fasting blood glucose sample, with the subject fasted overnight
2) Consumption by the subject of either 75g of oral glucose or 410ml of Lucozade
3) Collection of a further blood glucose sample 2 hours after consumption of the glucose

The World Health Organisation has published guidelines on reference ranges for blood glucose measurements of venous plasma in the diagnosis of DM.[175] Subjects are diagnosed as normal if fasting plasma glucose is <6.1mmol/l, or if fasting plasma glucose is <7.0mmol/l and 2 hour plasma glucose is <7.8mmol/l.

4.4 Methods

The study was planned as a case-control observational study. Ethical approval for the study was obtained from the North West 2 Research Ethics Committee – Liverpool Centre (reference number 09/H1005/68), and was registered with the RLUH Research Development and Innovation department (R&D No 3853).

Once subjects agreed to take part, and met the inclusion/exclusion criteria, a consent form was completed. One eye per subject was included in the study. This was to minimise selection bias as subjects with severe disease were more likely to have only eye that met criteria, whilst those without disease were more likely to have both eyes suitable for inclusion. If both eyes were eligible then one was selected if it had not previously received any treatment, had the clearer media or

had the better corrected visual acuity. Clinical care was not compromised, with subjects receiving appropriate treatment as required, including regular review by senior staff.

All subjects recruited underwent the following procedures during the study:

- best corrected visual acuity and contrast sensitivity
- blood pressure, serum lipid profile, serum glycated haemoglobin
- clinical examination
- multifocal electroretinography
- oscillatory potentials
- microperimetry
- colour photography
- optical coherence tomography
- fundus fluorescein angiography

4.4.1 Best corrected visual acuity (BCVA) and contrast sensitivity (CS) protocol

BCVA and CS were assessed by an accredited optometrist using our standard clinical protocol.

For BCVA, subjects initially underwent subjective refraction. An ETDRS chart was placed at 4 metres and each eye was tested individually in a well-lit room. Standard illumination was used for the chart. The number of letters on the chart that were read was recorded. BCVA was calculated as the number of letters read at 4 metres plus 30 and recorded as the equivalent to the numbers of letters read at 1 metre. . A score of 85 is equivalent to 6/6 on a Snellen chart.

A Pelli-Robson chart located at 1m was used for assessment of CS. In a well-lit room, each eye was tested individually with the fellow eye covered. The chart comprised 48 letters (eight rows of six letters) of equal size but with decreasing contrast of 0.15 log units for every group of three letters. The number of letters read by the subject was recorded.

4.4.2 Blood pressure, serum cholesterol level, serum glycated haemoglobin protocol

Blood pressure (BP) was recorded (in mmHg) as an average of four recordings taken from the same arm, two minutes apart. Systolic and diastolic BP were recorded separately for analysis.

Blood serum analysis comprised total cholesterol level in all subjects and glycated haemoglobin (HbA1c) in subjects with DM. As described above, healthy controls underwent OGTT to confirm absence of DM.

Total cholesterol was recorded in mmol/l and HbA1c as proportion of haemoglobin molecules that were glycosylated (%). All blood analyses were performed in the biochemistry laboratory of RLUH. These blood tests were repeated at subsequent visits.

4.4.3 Clinical examination

A clinical history was taken at baseline consisting of age of subject in years, age at diagnosis of DM, duration of DM, gender, smoking status, previous diabetic retinopathy treatment, previous medical history and medication history. I recorded the number of antihypertensive and lipid lowering agents prescribed, the use of insulin and the number of oral hypoglycaemic agents.

Both eyes of each subject were then maximally dilated with 1% tropicamide and 2.5% phenylephrine for examination of the eye and for the remaining investigations. Clinical examination was performed by me on a slit-lamp biomicroscope using a 60D non-contact lens to assess the fundus and record the diabetic retinopathy status and other retinal pathology, such as previous retinal laser.

4.4.4 Multifocal electroretinography (mfERG)

A standard departmental protocol was utilised for performing mfERG on a Retiscan® (Roland Consult, Brandenburg, Germany). Three Liverpool thread electrodes were used for recording and placed around the eye being tested as follows:

i) Ground electrode – forehead

ii) Reference electrode – lateral canthus

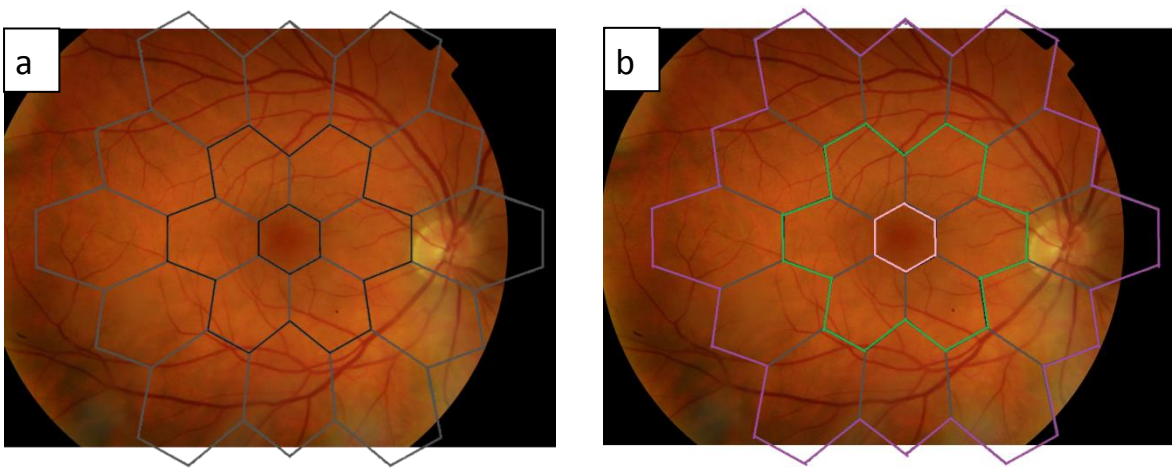
iii) Active electrode – lower conjunctival fornix

MfERG was performed as per the local protocol by accredited clinical electrophysiologists.[137] A 19 hexagon stimulus array covering 43° was presented on a 60 Hz CRT, and the stimulus was buffered with 4 blank filler frames so that the time between each step of the m-sequence was 83ms. Subjects focused on a central cross. Luminance of the mfERG was 110candela/m^2. Filter settings for the mfERG was 2-200Hz, with digital signal processing (DSP) between 1-50Hz.

The subject fixates on the stimulating monitor. The stimulus consists of an array of 19 hexagons covering an area of 40^o centred on the fovea. These hexagons alternate between light and dark flashes. The sizes of the hexagons are scaled according to the density of retinal response, and so get larger as they extend peripherally.

The 19 responses were then averaged into three concentric rings containing one, six and twelve hexagons respectively (radii being 2.65°, 2.65-10.75°, 10.75-21.75°) (Figure 4.1). The group waveforms were analysed and described by the central ring amplitude density (nV/deg^2) and latency (ms). The central hexagon covered the fovea and was used for analysis.

Figure 4.1: a) Representation of the hexagonal arrays and the areas of the retina stimulated; b) the hexagons comprising the three rings: central (pink), middle (green) and purple (outer). The central ring is used for analysis of central macular function



4.4.5 Oscillatory potentials (OPs)

Photopic OPs were gathered as per our local protocol and performed to ISCEV standards.[176] A full-field ERG (Ganzfeld) stimulation (3cd.s/m^2 on 30 cd/m^2) was used. Bandpass was set at 75-300Hz and artefact reject at 200μV. Sum amplitude (μV) of the four peaks generated was analysed. Preliminary analysis revealed that there was no significant difference between groups with respect to the implicit time of the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} peaks and so only the findings of the 1^{st} peak have been reported for implicit time (ms).

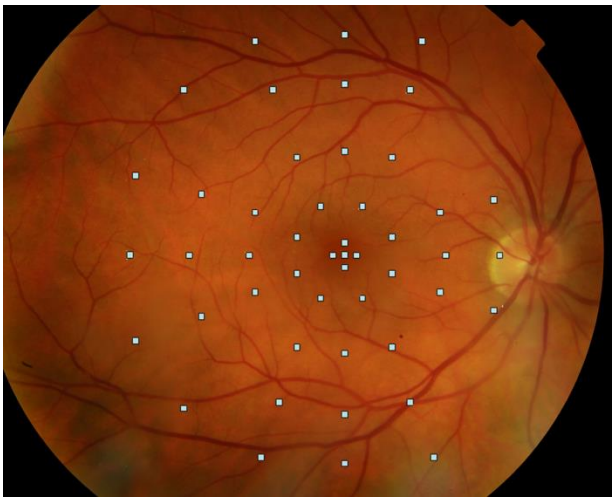
Electrophysiology testing was conducted with silver thread electrodes located at forehead and just beside outer canthi for ground and reference electrode respectively.

4.4.6 Microperimetry (MP)

MP was performed on all subjects using an automatic fundus-related perimeter (MP1 Microperimeter, Nidek Technologies, Padova, Italy). For the purpose of my study a protocol was specifically designed based upon the 19 hexagon mfERG array and the OCT grid pattern. The following parameters were set: a fixation target consisted of a red cross, 2^o in diameter; a white monochromatic background was set at 4 asb; a stimulus size of Goldmann III, with a projection time of 200ms; a customized radial grid of 48 points covering an area of 32^o in diameter centred on the fovea. A 4-2-1 double-staircase strategy was used with a starting stimulation of 10dB. The stimulus was projected onto a predefined retinal focus and the automated eye tracker ensured the same positions were stimulated at different light intensities. The results of the stimuli were then projected onto a digital colour photograph.

The central five points were averaged to give a mean sensitivity (dB) of foveal function (Figure 4.2). Central fixation of the subject was also recorded through automated eye tracking prior to assessing retinal sensitivity and provides a measure of fixation stability. For the purpose of my research if <75% of stimuli were seen in the central 4^o of fixation then the test was recorded as being unstable and not suitable for analysis.

Figure 4.2: Example of the output generated from the MP1 showing the foci of stimulus and the sensitivities recorded; the central five points were used for analysis of foveal function



4.4.7 Colour photography (CF)

All colour photographs were captured by an accredited image technician using a TopconTRC-50 X camera (Topcon Great Britain Ltd, UK). The protocol for the seven field photographs is described in Chapter3, Section 3.3.

CF was used to assess retinopathy status and to monitor disease progression. Images were stored on the ImageNet programme accessible on the local network. I analysed all CF images to confirm retinopathy status.

4.4.8 Optical coherence tomography (OCT)

OCT images were captured using a Spectralis HRA+OCT camera (Heidelberg Engineering, Heidelberg, Germany). The following settings were used:

i) Scan area = 20mm by 15mm; centred on the fovea

ii) 37 B-scans per scan area, spaced approximately 50µm apart

iii) 537 A-scans per B-scan

iv) Automatic-real time (ART) = 16

Two sets of images were produced. Firstly cross-sectional images of the retina corresponding to the B-scans were viewed individually. Secondly, the cross-

sectional images were then collated and the gaps filled to create a volume scan. An ETDRS grid, composed of 3 rings measuring 1000µm, 3000µm and 6000µm in diameter, was superimposed onto the volume scan and used to determine thickness of the retina within each ring structure. Though the Heidelberg software generates the ETDRS grid and automatically determines the foveal centre, I assessed the location of the grid and manually readjusted it if necessary.

For the purpose of my research I am using central subfield thickness (CSFT) as a measure of macular thickness as it corresponds to mean thickness within the central 1000µm of the macula. In Chapter 5, I describe how I have used this measurement to compare OCT to MP and mfERG.

4.4.9 Fundus fluorescein angiography (FA)

This investigation was also performed by accredited image technicians on the HRA+OCT camera. A local protocol was used for capturing FA images. Thirty degree images were taken based on the same seven fields described for CF in Chapter 3, Section 3.3. Following an intravenous injection of 3ml of 25% sodium fluorescein over 3 seconds, images were taken of both the study eye and the fellow eye (Table 4.2)

Images captured during transit phase allow for assessment of FAZ and the perifoveal capillary network. Stereo images of the macula were used to assess leakage from microaneurysms whilst peripheral images were assessed for signs of diabetic retinopathy (DR). Late phase images were assessed for the presence of cystoid leakage and dye accumulation at the fovea.

Table 4.2: Summary of the images captured during FA. F1 = images centred on the optic disc; F2 = images centred on the fovea; F3-F7 = images focused on the peripheral retina

Phase	Time	Image Capture	Eye
Transit	0-30s(approx) 30-40s(approx) 40-60s	F2 every 1-2 seconds F2 single images F2 single images	Study Fellow Study
Mid	1-5min	F1+F2 stereo F1+F2 stereo F3-7 F3-7	Study Fellow Study Fellow
Late	5 min 10 min	F1+F2 stereo F1+F2 stereo	Both Both

I manually assessed the transit phase images to determine the clearest image for assessing the FAZ. Once the FAZ was outlined I measured its greatest diameter (GLD) in microns and used this value to determine the presence of ischaemic macular changes. A GLD greater than 1000 µm with disruption of the perifoveal capillary network was used for diagnosis of ischaemic maculopathy.

4.5 Subject recruitment and follow up

Subjects were recruited into four different groups:

I. Healthy controls
II. Diabetic controls (subjects with DM but no DR)
III. Early diabetic maculopathy
IV. Sight-threatening maculopathy (CSMO +/- ischaemic maculopathy)

Groups 2-4 were then followed up at six months and 12 months and all above the investigations were repeated at each visit. Subjects exited the study if they

completed the 12 month visit or if intraocular treatment was required for maculopathy.

The sample size for each group had been estimated, by medical retina specialist and statistician, based on the following outcomes:

- mean value of retinal sensitivity (dB) at 12° on MP
  (mean difference of 4.5 (±3.5) dB judged clinically relevant)
- mfERG central ring implicit time
  (mean difference of 3 (±1) ms judged clinically relevant)
- summed OP amplitude
  (mean difference of 4 (±6) μV judged clinically relevant)
- mfERG central ring amplitude
- OP 1st peak implicit time

To detect these differences statistically with 90% power using ANOVA test and a paired t-test with $p<0.05$, a minimum sample size of 26 patients was calculated. Taking into account potential dropouts, it was decided to recruit 30 subjects to each group.

4.6 Study outcomes

4.6.1 Primary outcomes

The primary outcomes of my study are based around changes in function at the cross sectional analysis with regards to severity of maculopathy. These outcomes are:

- Is MP sensitivity reduced with increasing severity of maculopathy?
- Is mfERG central ring amplitude reduced with increasing of severity of maculopathy?
- Is mfERG IT prolonged in the presence of maculopathy?
- Is OP sum amplitude reduced with increased severity of maculopathy?
- Is OP IT prolonged in the presence of maculopathy?

4.6.2 Secondary outcomes

- Are BCVA/CS reduced with increased severity of maculopathy?
- Is there increased CSFT in maculopathy?
- Is severity of maculopathy associated with change in central macular function over time?
- Is there an association between functional tests and subjects who required treatment for maculopathy or were diagnosed with CSMO or ischaemic maculopathy?

4.7 Statistical analysis

Data were recorded on an Excel Spreadsheet. All data quality control checks and all statistical analyses were performed on SPSS for Windows version 22 (SPSS Inc, Chicago, USA), under the guidance of the departmental biostatistician (Dr G. Czanner, CStat), with the aim to answer the research questions as stated in Chapter 3. Subjects were given their own unique study code and details were kept confidential in accordance with the Data Protection Act 1998 and the Research Governance Framework for Health and Social Care 2010.

4.7.1 Baseline data analysis

I initially performed the Kolmogrov-Smirnov test to determine whether the data were normally distributed. If data was normally distributed, in order to evaluate potential confounders across 5 groups, I used the ANOVA with 1 factor test to determine statistical significance; if not normally distributed I used the Kruksal-Wallis test. I used Levene's test for analysis of homogeneity of variance.

4.7.1.1 Demographic data analysis

For continuous variables I calculated the mean and standard deviation of the demographic data of each group. The continuous variables include age, duration of DM, serum cholesterol level, serum HbA1c level, and systolic and diastolic BP.

For discontinuous data I assessed median and interquartile range. Discontinuous variables included number of antihypertensive agents used and number of oral hypoglycaemic agents.

For categorical data, such as gender, retinopathy grading, smoking status and insulin use, I calculated percentage of subjects within each category. For retinopathy grading I used Fisher's exact test of association and Pearson chi-square test of association for comparison of subjects with maculopathy.

For the demographic data, $p<0.05$ was deemed significant for inter-group comparisons.

4.7.1.2 Structural and functional data analysis

I calculated the mean and standard deviation for the following variables:

- BCVA
- CS
- CSFT on OCT
- mfERG central ring amplitude
- mfERG central ring implicit time
- OP sum amplitude
- OP 1st peak implicit time
- MP central ring sensitivity

The differences in retinal function across groups were analysed by ANOVA with adjustment for possible confounders via ANCOVA. To accomplish a family-wise error rate of 0.05 for our five primary outcomes Bonferroni correction was used by comparing the five p-values with significance level of 0.01. After ANCOVA, post-hoc comparisons between the groups were performed using the two-sample t-tests and multiple comparison method of Sidak. Such p-values were then compared with significance level of 0.01. For other outcomes, such as BCVA, CS and OCT CFT, $p<0.05$ was deemed to be significant but should be interpreted with caution as they are not primary outcomes. Results are presented in a tabular format.

4.7.2 Longitudinal analysis

Only subjects who completed the month 6 and month 12 visits were included in the analyses. Firstly I performed ANOVA to assess for confounders to change in functional assessment. Variables analysed were age at baseline, serum cholesterol,

serum HbA1c, systolic blood pressure, diastolic blood pressure, BCVA and CS. $P<0.05$ was deemed significant.

I used ANOVA, with adjustment for possible confounders via ANCOVA, to compare subjects who completed the study to those who exited the study. I calculated the mean and standard deviation. $P<0.05$ was deemed significant.

To determine change in retinal function over 6 months and 12 months, I analysed mean baseline values and mean change in function for the data as a whole at both time points. For this I used ANCOVA with adjustment for baseline function. Along with mean I calculated standard deviation and range. For change in function I analysed number of subjects in whom function had improved, worsened or stayed the same to highlight trend. $P<0.05$ was deemed to be significant.

In the intra-group comparison of subjects with DM I performed Wilcoxon test to assess mean change in function from baseline. Once again I analysed number of subjects in whom function had improved, worsened or stayed the same. I also performed the paired t-test to compare subjects with maculopathy. For both tests $P<0.05$ was deemed to be significant.

4.7.3 Analysis of association

To further evaluate the diagnostic potential of these tests I performed the following analyses. For each test of visual function I found the critical quartile value that represented reduced function. For BCVA, CS, MP, mfERG amplitude and OP sum amplitude I used the lower quartile; for OP IT and mfERG central ring IT I used the upper quartile. The quartiles were calculated from all subjects with DM.

In the cross-sectional analyses I divided the subjects with DM into those with sight-threatening maculopathy and those who did not have sight-threatening maculopathy. In the longitudinal analyses I divided the subjects into those who received treatment and those who did not receive treatment within 6 and 12 months of entering the study. I then tested the association between the two categories for each analysis with the Fisher exact test. I calculated the confidence intervals for probability of either having CSMO or ischaemic maculopathy in the

cross-sectional analysis, or requiring treatment in the longitudinal analysis, using the Binomial confidence interval (Clopper-Pearson formulae).[177]

Using the same statistical method I performed a further analysis to determine whether combining two investigations demonstrated a stronger association for the two groups mentioned above. The investigations analysed were those related to visual function, i.e. BCVA, CS, MP, mfERG and OP.

4.8 Summary

In this chapter I have demonstrated the investigative techniques that will be undertaken to answer the research questions. In the subsequent chapters I will present the results of my analysis following the methods described above. However, before I present the results, I demonstrate in Chapter 5 how I have correlated the structural assessments (ETDRS grid) with the functional assessments (mfERG hexagonal array and MP stimulus foci) as a multimodal imaging methodology.

Chapter 5 Multimodal analysis of retinal structure and function

Central to the work that I will present in my thesis is the use of a multimodal approach to the assessment of retinal function using mfERG and MP and structure using OCT, diverse platforms that have been developed independently. Each uses a map or grid to allow a topographical segmentation of the macula. In this chapter I describe an analysis of the published anatomical measurements of retinal structures to allow the development of co-registration of the platform maps and subsequent multimodal analysis of the pathology of diabetic retinopathy.

5.1 Measurement of structures within the retina in micrometres

Anatomical studies have estimated values of the sizes of various structures within the retina, some of which form the macula. These structures include:

- Fovea
- Foveola
- Macula
- Optic disc
- Distance between centre of fovea and temporal edge of optic disc
- Distance between centre of fovea and centre of optic disc

5.1.1 Fovea, foveola and macula

It has been shown that the diameter of the foveola ranges from 300 to 400 µm, with the commonest stated value as 350 µm.[174] The diameter of the fovea ranges from 1200 to 1800 µm, with the commonest value estimated as 1500 µm.[174] The macula ranges from 5000 to 6000 µm, with a diameter of ~5500 µm accepted universally.[173,174,178]

5.1.2 Optic disc

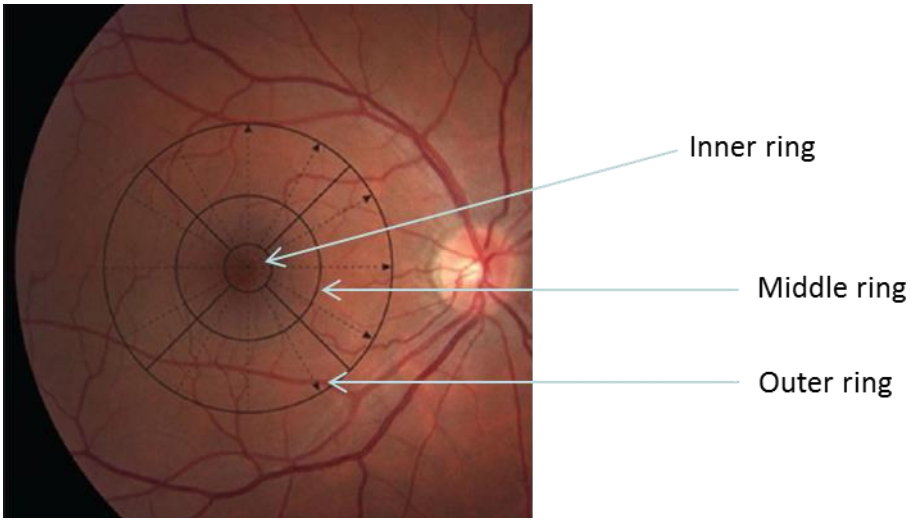
Other distances that have been analysed include the relationship of the fovea to the optic disc. The horizontal diameter of the optic disc may range from 900 µm to 2600 µm, though the commonest values are estimated at 1500 to 1800 µm.[173,179,180]

In addition the distance from the centre of the fovea to the temporal edge of the disc ranges from 3000 to 3800 µm, i.e. the outer edge of the macula does not intersect the temporal edge of the disc.[180,181] Another value is from the centre of the disc to the centre of the fovea, which ranges from 3750 to 4500 µm.[180]

5.2 The ETDRS grid

As described in Chapter 3, the Early Treatment Diabetic Retinopathy Study (ETDRS) outlined criteria for CSMO. To assess the location of lesions relative to the centre of the fovea the investigators utilised a grid that could be placed on the colour photograph and FA, initially applied manually to colour 35mm film and then later to digital images. This circular grid comprised three rings of diameters of 1mm, 3mm and 6mm (Figure 5.1). These diameters were chosen because the inner ring (1mm) would identify any lesions within 500µm of the centre of the fovea; the middle ring (3mm) would identify any lesions within one disc diameter of the fovea; and the outer ring (6mm) would identify any lesions within the macula.

Figure 5.1: Schematic to show the three rings of the EDTRS grid centred on the foveal centre



The ETDRS grid has become an essential component of clinical practice, as it is used routinely in the analysis of images of the macula generated by OCT. Thus any study of diabetic maculopathy is likely to use this tool in the analysis of its data. Therefore I will be using the ETDRS grid in the analysis of OCT images in my data.

5.3 Applying multifocal electroretinography and microperimetry to retinal analysis

As described above, the ETDRS grid is based upon a measurement in millimetres. However the mfERG hexagonal and MP patterns are measured in degrees from the foveal centre. Therefore, to compare these investigative techniques, a conversion rate is required to convert millimetres to degrees. Unfortunately neither the mfERG nor the MP software provides a ratio of degrees to millimetres to allow cross calibration.

5.3.1 Measurement of structures in the retina in degrees

Wolff's Anatomy states that the fovea has a diameter of 1850 µm and represents 5^{o} of the visual field.[173] It also states that the macula is 5500µm in diameter and covers 15^{o} of visual field. This suggests that 1^{o} is equivalent to ~370 µm. It also states that the foveola is 350 µm in diameter and covers 1^{o} of visual field. However, Drasdo and Fowler (1974)[179] state that "one degree of visual angle is equal to 288 µm on the retina without correction for shrinkage". They also state that centre of the fovea is 3400 µm or 11.8^{o} from the temporal edge of the disc. This means 1^{o} equates to just over 288 µm. Therefore 1^{o} of visual angle may range from 288 µm to 366.67 µm. Due to the variations in the distances in the retina and equivalent degrees of visual angle, there may be discrepancies when comparing the ETDRS ring to the MP and mfERG hexagonal grids.

5.3.2 Combining MP grid with ETDRS ring

To determine a suitable conversion factor from microns to degrees I compared in one subject the MP grid as generated by Nidek's MP1 software to the ETDRS grid generated by the Heidelberg Spectralis system when capturing infra-red reflectance images of the macula during OCT acquisition.

Figure 5.2 shows the infra-red reflectance image of a subject with early diabetic maculopathy. The ETDRS grid has three rings of the standard diameters and has been generated automatically by the supporting software. I will use this image to compare with the MP grid for the same subject.

Figure 5.2: Infra-red reflectance image of subject demonstrating the position of the ETDRS grid centred on the fovea

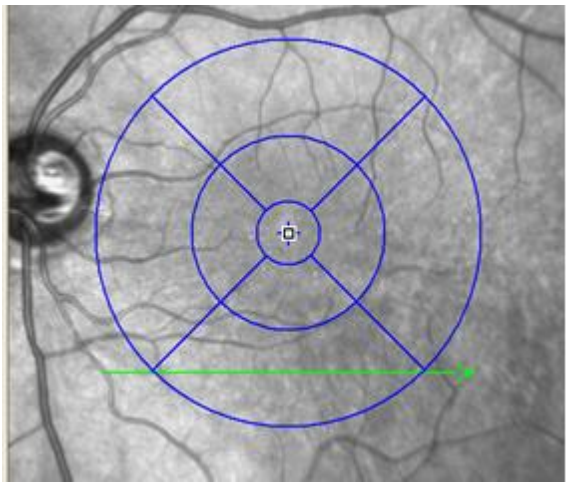
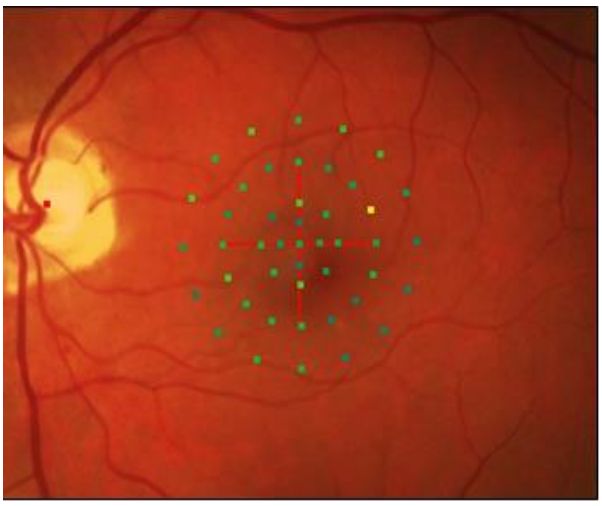


Figure 5.3 is the MP for the same subject. I have selected a 12^{o} grid with 45 points for comparison as it is a grid pattern that is most commonly reported in literature and the points are located closely together allowing for more accurate assessment of distance measurements.[165,167,182]

Figure 5.3: MP grid centred at the fovea for the same subject as Figure 5.2 demonstrating the foci of retinal sensitivities that were assessed.



The rings on this 12^{o} MP grid comprise of the following diameters:

- inner ring = 2^{o} in diameter
- 2^{nd} ring = 4^{o} in diameter
- 3^{rd} ring = 8^{o} in diameter

- outer ring = 12^{o} in diameter

As demonstrated previously 1^{o} of visual angle may vary between 288 µm and 366.67 µm. For ease of calculations I will compare the position of the ETDRS ring within a 12^{o} MP grid if 1^{o} was equivalent to 250 µm and 350 µm. Table 5.1 calculates the MP ring diameters if 1^{o} was equivalent to 250 µm and 350 µm.

Table 5.1: Diameter of the MP rings converted from degrees to microns if 1^{o} was equivalent to 250 µm and 350 µm

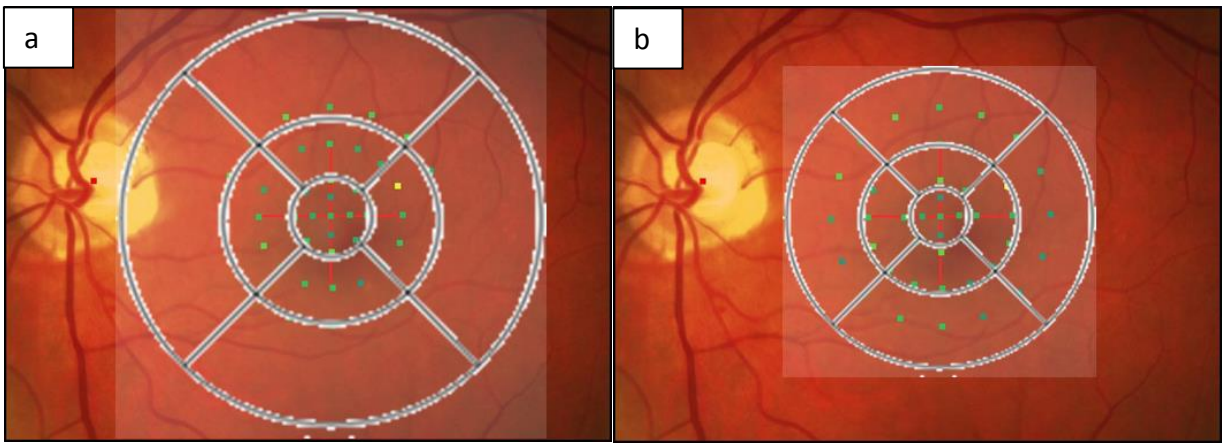
Ring	If 1^{o} = 250 µm	If 1^{o} = 350 µm
Inner	500 µm	700 µm
2^{nd}	1000 µm	1400 µm
3^{rd}	2000 µm	2800 µm
Outer	3000 µm	4200 µm

Using these ring calculations I calculated the location of the ETDRS grid to the 12^{o} MP grid shown in Figure 5.3. I then compared the results to the grid shown in Figure 5.2. In particular I looked at the outer ring of the ETDRS grid and its relation to the optic disc and the superotemporal retinal vascular arcade.

Figure 5.4a demonstrates the position of the ETDRS grid based upon the MP grid if 1^{o} is equivalent to 250 µm. The inner ring of the ETDRS grid would overlay the points on the 2^{o} MP ring. The outer ring would transect the optic disc and the superotemporal retinal vascular arcade. Compared to Figure 5.2, the grid would cover a larger area than expected. This suggests that 1^{o} is more than 250 µm

Figure 5.4b demonstrates the position of the ETDRS grid based upon the MP grid if 1^{o} is equivalent to 350 µm. Though the outer ring is now within the arcade, it no longer crosses the edge of the optic disc as in Figure 5.2. Therefore the ETDRS now appears to cover a smaller area than expected and so 1^{o} must be less than 350 µm.

Figure 5.4: Comparison of the position of the ETDRS grid to a 12^{o} MP grid if (a) 1^{o} is equivalent to 250µm and if (b) 1^{o} is equivalent to 350µm

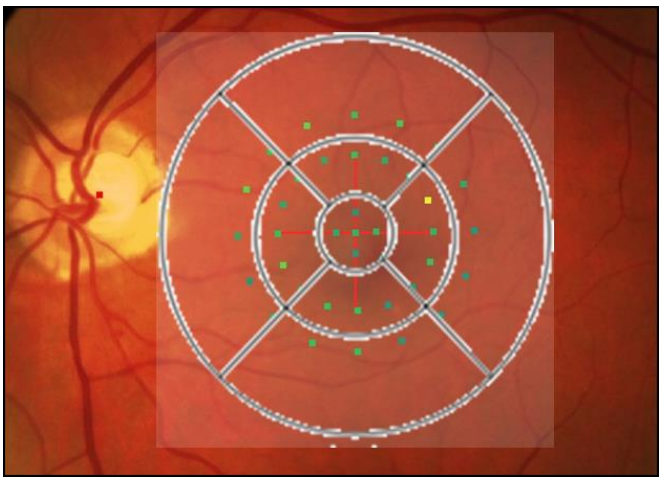


Therefore it appears that 1^{o} lies between 250 and 350 µm. I compared Figures 5.2-5.4 and noted that the outer ring in Figure 5.2 lay in between the outer rings of Figures 5.3 and 5.4.

I then calculated the distance between the centre of the fovea and the centre of the optic disc using the in-built measurement tool on OCT which measured 3550 µm. The customised 32^{o} MP grid used in my study, as seen in Chapter 4, had a focus corresponding to the centre of the disc. This focus was at 12^{o} from the centre of the fovea. Thus 1^{o} equates to ~296 µm which is not dissimilar from the 288 µm quoted by Drasdo and Fowler.[179]

For ease of calculation I used a value of 1^{o} = 300 µm and returned back to the 12^{o} MP grid. I repositioned the ETDRS grid over the 12^{o} MP grid for the subject in Figure 5.3. The result can be seen in Figure 5.5 and shows the ETDRS grid is positioned closely to that in Figure 5.2. Having determined that 1^{o} = 300 µm, I compared the placement of the EDTRS grid to the customised 32^{o} MP grid and repeated the process for the first five subjects in my study to check the credibility of this calculation. This confirmed that 1^{o} equates closely to 300 µm.

Figure 5.5: Comparison of the position of the ETDRS grid to a 12^{o} MP grid if 1^{o} is equivalent to 300µm



Therefore I have used a conversion of 1^{o} to 300µm when comparing the central ring of the customised 32^{o} MP grid to the ETDRS grid used for OCT measurements.

5.3.3 Comparing mfERG to ETDRS grid and MP grid

The rapid 19 hexagonal pattern developed in Liverpool and utilised in performing mfERG, as described in Chapter 4, is essentially composed of 3 rings made up of four different hexagonal structures (Figure 5.6).

Figure 5.6: Hexagonal pattern of multifocal electroretinogram showing the 19 hexagons

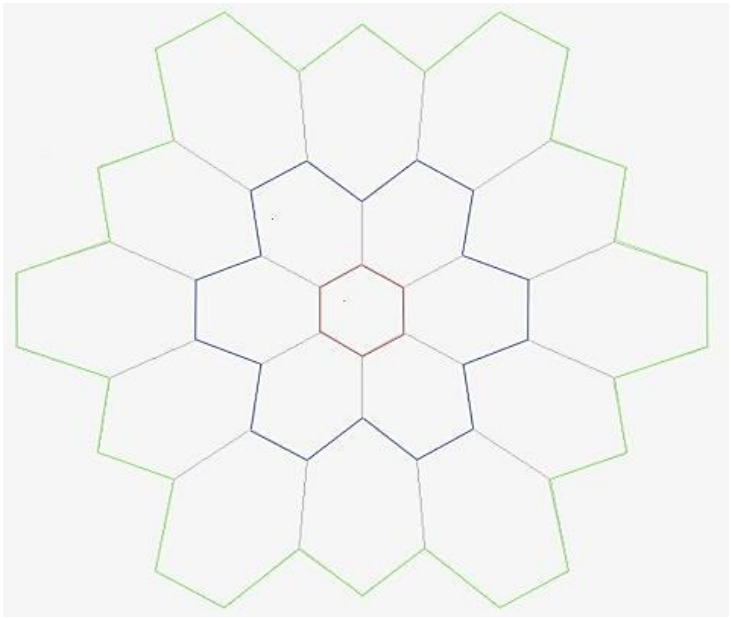


Figure 5.6 shows that the hexagonal pattern can be considered to be composed of three rings: an inner ring, middle ring and outer ring. The central ring comprises a single hexagon (Figure 5.7a). The middle ring is composed of six hexagons of equal dimensions (Figure 5.7b). The outer ring is composed of 12 hexagons alternating between two hexagonal structures (Figure 5.7c & d).

These sizes of these hexagons are shown in Figure 5.7 and are measured in degrees. Using the previously calculated conversion of 1^{o} to 300µm the sizes of the hexagons, in microns, are described in Table 5.2.

Figure 5.7: Hexagons of the mfERG grid to show their location and the measurements acquired: a) inner ring, b) middle ring, c) &d) outer ring

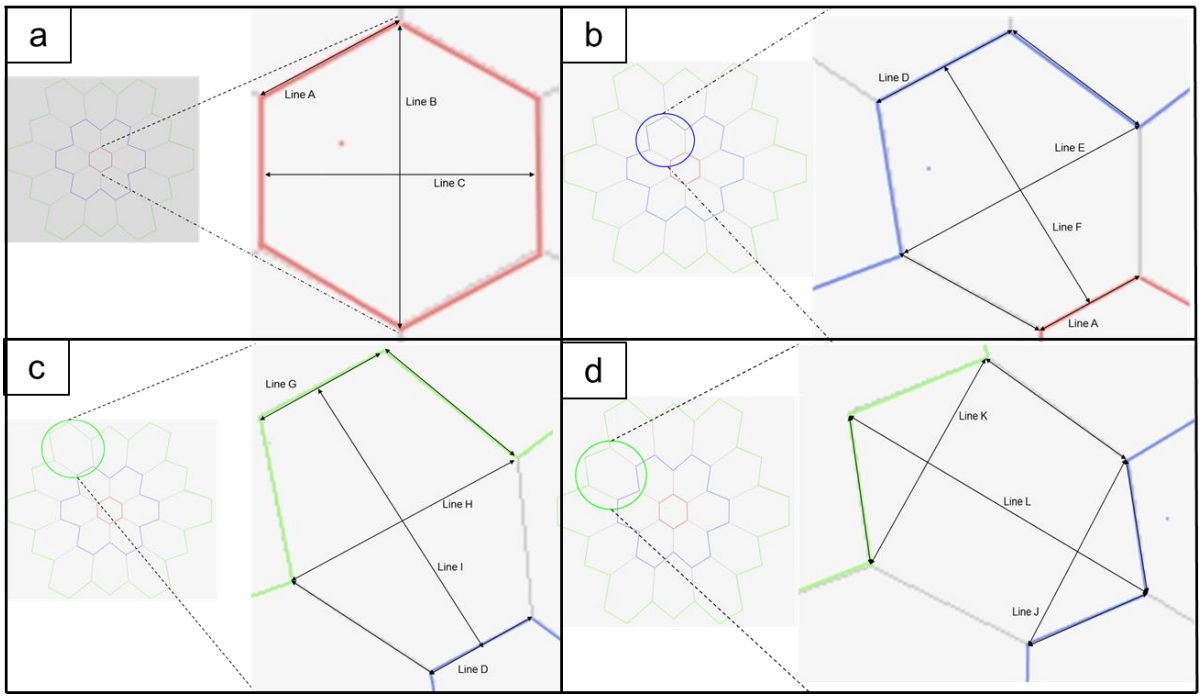


Table 5.2: Measurements of the lengths marked in figure 5.7 in both degrees and microns using a conversion of 1^{o} to 300µm

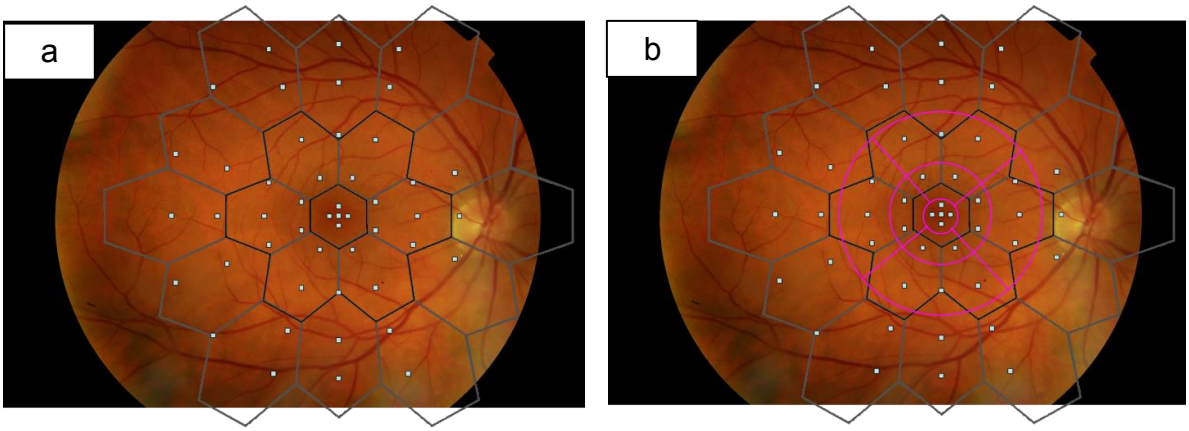
Hexagon	Line	Degrees	Microns
Inner (Figure 5.7a)	Line A	3.2	960
	Line B	5.4	1620
	Line C	6.4	1920
Middle (Figure 5.7b)	Line D	4.2	1260
	Line E	7.6	2280
	Line F	8.2	2460
Outer 1 (Figure 5.7c)	Line G	5.4	1620
	Line H	9.6	2880
	Line I	11.8	3540
Outer 2 (Figure 5.7d)	Line J	7.4	2220
	Line K	8.3	2490
	Line L	11.4	3420

Using the calculations in Table 5.2, it is possible to project the 32^{o} MP grid onto the hexagonal grid for the mfERG (Figure 5.8a) for comparison. In Figure 5.8b, I present a schematic that combines the ETDRS grid, MP grid and mfERG hexagonal pattern.

5.4 Combining analysis of the MP, mfERG and OCT

As can be seen in Figure 5.8, the central five loci of the MP grid sit within the central ring of the ETDRS grid and the central hexagon of the mfERG. Also, the central ETDRS ring sits within the central mfERG hexagon. Therefore, to compare these investigative modalities, I will use the mean of the five central loci of the MP grid, the central ring of the ETDRS grid for CSFT and the central hexagon of the mfERG.

Figure 5.8: Schematics combining the different investigative modalities a) mfERG hexagonal pattern and MP grid; b) mfERG hexagonal pattern, MP grid and ETDRS ring



A larger version of Figures 5.8a and 5.8 b are presented in Appendices 1 and 2 at the end of this thesis.

5.5 Summary

In this chapter I have demonstrated how to convert degrees of visual field into distances in microns. I have subsequently demonstrated how this conversion allows for the comparison between structural and functional assessments of macular function. In the next chapter I present the cross-sectional analysis of the baseline data.

Chapter 6 Results – cross-sectional

In this chapter I will present the results of the baseline data. I will describe the recruitment of subjects to my study and the cross-sectional results of the functional assessments of the central macula in subjects with diabetes mellitus (DM).

6.1 Recruitment

In Chapter 4 I described the eligibility and exclusion criteria of my study. In brief, subjects with DM were recruited into three groups: diabetic controls (no diabetic retinopathy); early maculopathy (presence of diabetic maculopathy not meeting criteria for clinically significant macular oedema (CSMO)); sight-threatening maculopathy (presence of CSMO with or without ischaemic macular changes). A healthy control group comprised subjects without DM.

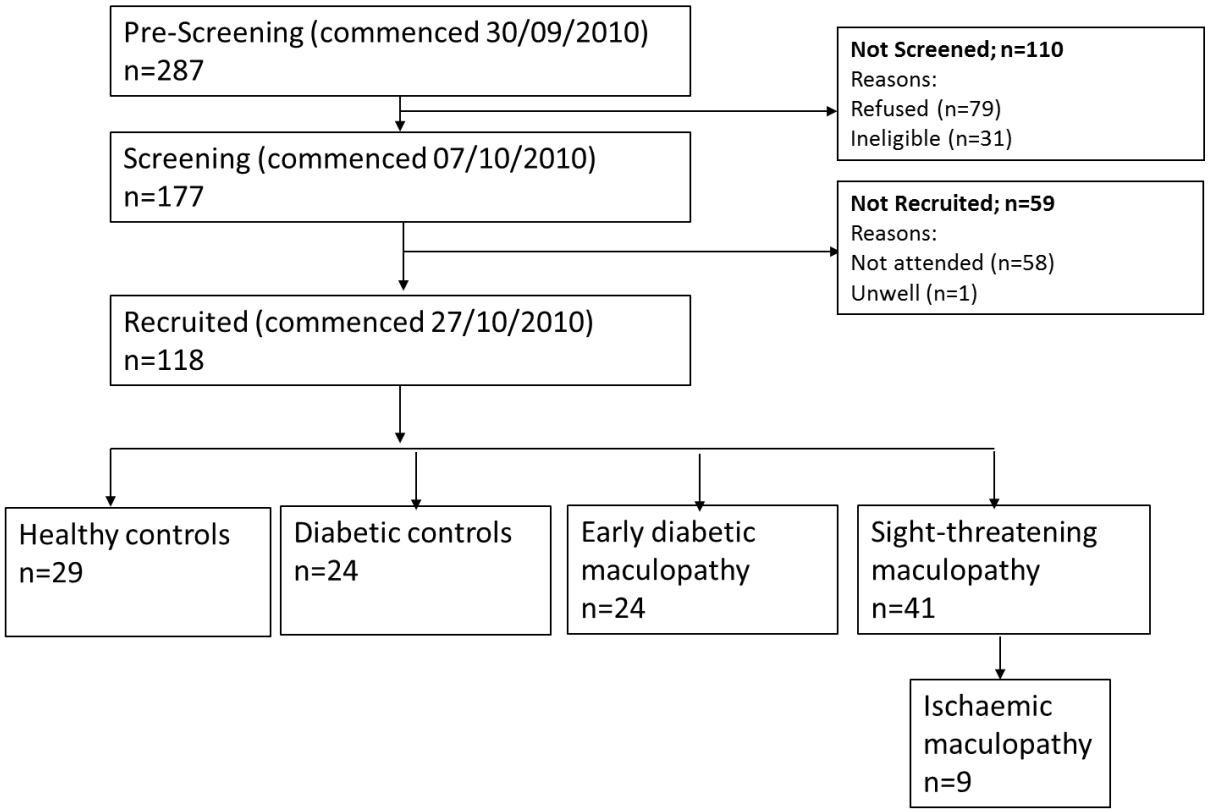
Subjects were recruited prospectively from a single centre (St Paul's Eye Unit) and were recruited in two phases. During the first phase, pre-screening commenced on 30th September 2010, and screening the following week. Pre-screening comprised of determining whether subjects met the inclusion/exclusion criteria and so would be eligible to take part in the study. Subjects were provided with a patient information leaflet. Screening comprised of determining whether inclusion criteria were met and whether the subject was willing to take part in the study.

Recruitment commenced on 27th October 2010, with the last subject recruited in phase 1 on 5th December 2012. A second phase of recruitment commenced screening on 7th August 2013 and completed recruitment on 3rd June 2015.

Figure 6.1 is a consort diagram that summarises the recruitment of subjects to my study and the groups to which they were recruited:

i) Healthy controls n=29
ii) Diabetic controls n=24
iii) Early diabetic maculopathy n=24
iv) Sight-threatening maculopathy n=41
    a) Presence of ischaemic maculopathy n=9

Figure 6.1: Consort diagram summarising the pre-screening, screening and recruitment of subjects to my study and the groups to which they were allocated.



6.2 Demographic data

A total of 118 subjects were recruited into the study. Sixty eight (55.9%) were male. Only eight (6.8%) were pseudophakic. The majority were non-smokers (83.1%) with 14 (11.9%) active smokers and 6 (5.1%) ex-smokers.

Table 6.1 shows the mean age in years of the groups. Healthy controls were younger than the other groups, but this difference was only statistically significant compared to diabetic controls ($p=0.03$). There was no statistically significant difference between the groups with DM ($p>0.2$ for all comparisons).

Table 6.1: Comparison of age (in years) between the groups. The second column shows mean age, standard deviation (SD) and range for each group. The third column shows mean difference in age, 95% confidence limits (CL) and p value for intergroup comparison. *P<0.05 is deemed significant

	Mean age (yrs) (SD) Range	Mean difference in age (years) (95% CL) P value		
		Diabetic control	Early maculopathy	Sight-threatening maculopathy
Healthy control n=29	50.6 (12.6) 28-68	9.9 (0.8, 18.9) 0.03*	5.2 (-3.9, 14.2) 0.56	3.4 (-4.6, 11.3) 0.84
Diabetic control n=24	60.5 (11.2) 35-78		-4.7 (-14.2, 4.8) 0.71	-6.5 (-15.0, 1.9) 0.22
Early maculopathy n=24	55.8 (12.9) 27-78			-1.8 (-10.2, 6.6) 0.99
Sight-threatening maculopathy n=41	54.0 (12.2) 28-74			

6.2.1 Systemic disease

Nearly half of subjects with DM (49.2%) had a diagnosis of hypertension and over half (57.6%) had a diagnosis of hyperlipidaemia. Eight subjects with DM (9.0%) had

a diagnosis of peripheral neuropathy and 13 (14.6%) of cardio- or cerebrovascular disease.

Of those subjects with DM, just over half (52.8%) were on insulin as part of their medical management.

Diabetic controls had a statistically significant shorter mean duration of DM (7.3 years) as compared to those with early maculopathy (16.3 years, $p<0.01$) and those with sight threatening maculopathy (15.9 years, $p<0.01$) (Table 6.2). There was no significant difference in duration between both groups with maculopathy ($p>0.50$).

6.2.2 Serum glycated haemoglobin (HbA1c)

Of those subjects with DM, those without retinopathy had a significantly lower mean HbA1c (7.0%) compared to subjects with early maculopathy (8.7%, $p<0.01$) and subjects with sight threatening maculopathy (8.6%, $p<0.01$) (Table 6.3). There was no significant difference in serum HbA1c between the two maculopathy groups ($p>0.50$).

6.2.3 Serum cholesterol

There was no significant difference in serum cholesterol levels between any of the four groups ($p>0.50$) (Table 6.4). Healthy controls were less likely to be on lipid lowering agents (17%) as compared to subjects with DM (76%, $p<0.01$). However there was no significant difference between subjects with DM ($p>0.50$).

6.2.4 Blood pressure (BP)

Mean systolic BP (SBP) was significantly higher in the sight-threatening maculopathy group (134.0 mmHg) compared to healthy controls (123.7 mmHg, $p<0.05$) (Table 6.5). SBP in diabetic controls (133.1mmHg, $p>0.10$) and subjects with early maculopathy (133.7mmHg, $p>0.10$) was greater than healthy controls though this did not reach statistical significance.

There was no significant difference in diastolic BP (DBP) between any of the groups ($p>0.50$).

Similar to statin use, subjects with DM were more likely to be taking anti-hypertensive agents (63%) compared to healthy controls (10%, p<0.01). There was no significant difference between subjects with DM (p>0.50).

Table 6.2: Comparison of duration of DM (years) between the groups. Column 2 shows mean duration with standard deviation (SD) and range. Column 3 is an intergroup comparison of mean difference in duration with confidence limits (CL). *P<0.05 is deemed significant.

	Mean duration of DM (years) (SD) Range	Mean difference in duration of DM (years) (95% CL) P value	
		Early maculopathy	Sight-threatening maculopathy
Diabetic control n=23	7.3 (6.6) 0.5-25	9.0 (3.4, 14.5) <0.01*	8.6 (3.6, 13.5) <0.01*
Early maculopathy n=24	16.3 (9.5) 4-43		-0.4 (-5.3, 4.5) 1.00
Sight-threatening maculopathy n=40	15.9 (7.3) 0.3-30		

Table 6.3: Comparison of mean serum HbA1c (%) between the groups. Column 2 shows mean duration with standard deviation (SD) and range. Column 3 is an intergroup comparison of mean difference in duration with confidence limits (CL). *P<0.05 is deemed significant.

	Mean serum HbA1c (%) (SD) Range	Mean difference in HbA1c (%) (95% CL) P value	
		Early maculopathy	Sight-threatening maculopathy
Diabetic control n=23	7.0 (1.2) 5.1 – 9.7	1.7 (0.5, 3.0) <0.01*	1.6 (0.6, 2.7) <0.01*
Early maculopathy n=24	8.7 (1.6) 5.6 – 11.1		-0.1 (-1.2, 1.0) 1.00
Sight-threatening maculopathy n=41	8.6 (1.8) 5.6 – 13.7		

Table 6.4: Comparison of mean serum cholesterol (mmol/l) between groups. Column 2 is mean level with standard deviation (SD), range. Column 3 is comparison of mean difference (confidence limits (CL)). *P<0.05 is deemed significant.

	Mean serum cholesterol (mmol/l) (SD) Range	Mean difference in serum cholesterol (mmol/l) (95% CL) P value		
		Diabetic control	Early maculopathy	Sight-threatening maculopathy
Healthy control n=28	4.9 (0.9) 3.3 – 7.2	-0.4 (-1.1, 0.4) 0.76	-0.4 (-1.2, 0.3) 0.61	-0.2 (-0.9, 0.4) 0.92
Diabetic control n=23	4.5 (0.9) 3.0 – 6.0		-0.1 (-0.9,0.8) 1.00	0.1 (-0.6, 0.8) 1.00
Early maculopathy n=24	4.5 (1.1) 3.0 – 7.0			0.2 (-0.5,0.9) 0.99
Sight-threatening maculopathy n=41	4.6 (1.1) 2.2 – 6.8			

Table 6.5: Intergroup comparison of mean systolic blood pressure (mmHg). Column 2 shows mean duration with standard deviation (SD) and range. Column 3 is an intergroup comparison of mean difference in duration with confidence limits (CL). *P<0.05 is deemed significant.

	Mean systolic BP (mmHg) (SD) Range	Mean difference in systolic BP (mmHg) (95% CL) P value		
		Diabetic control	Early maculopathy	Sight-threatening maculopathy
Healthy control n=29	123.7 (17.5) 96-170	9.4 (-2.3, 21.1) 0.19	10.1 (-1.6, 21.7) 0.13	10.3 (0.1, 20.5) <0.05*
Diabetic control n=23	133.1 (14.3) 112-170		0.6 (-11.7, 12.9) 1.00	0.9 (-10.1, 11.9) 1.00
Early maculopathy n=24	133.7 (17.5) 111-168			0.3 (-10.5, 11.1) 1.00
Sight-threatening maculopathy n=41	134.0 (13.9) 109-168			

6.2.5 Ophthalmic history

Of those subjects with DM, 39 (43.8%) had prior peripheral retinal laser. Very few subjects had other ophthalmic conditions, with one subject suffering from primary open angle glaucoma, two with mild corneal pathology and two having had previous laser refractive surgery.

6.2.6 Retinopathy grading

With respect to retinopathy grading, 58.3% of subjects with early maculopathy had a grading of R1 and 41.7% a grading of R2. In subjects with sight-threatening maculopathy, these figures were reversed with 41.5% having a grading of R1 and 58.5% a grading of R2. This was not found to be statistically significant on both Pearson chi square test of association (2-sided, $p=0.19$) and Fisher's exact test of association (2-sided, $p=0.21$).

6.3 Assessments of central macular function and structure

6.3.1 Best corrected visual acuity (Table 6.6, Figure 6.2)

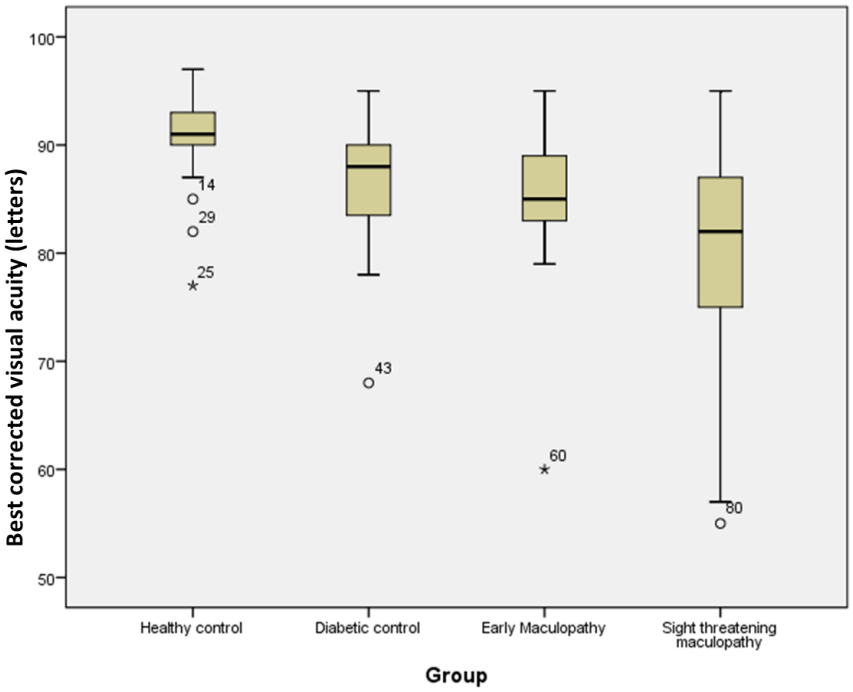
Refraction protocol best corrected visual acuity (BCVA) differed significantly across groups ($p<0.05$, ANOVA F-Test). Mean BCVA was significantly reduced in subjects with early maculopathy (84.8 letters, $p<0.05$) and subjects with sight-threatening maculopathy (80.6 letters, $p<0.01$) as compared to healthy controls (90.6 letters) (Table 6.6). There was no significant difference between healthy and diabetic controls (86.8, $p>0.1$).

Mean BCVA was significantly reduced in subjects with sight-threatening maculopathy compared to diabetic controls ($p<0.02$). There was no significant difference between diabetic controls and subjects with early maculopathy ($p>0.5$), nor between subjects with maculopathy ($p>0.10$).

Table 6.6: Intergroup comparison of best corrected visual acuity without and with correction for age. P value represents age corrected comparison. *P <0.05 is deemed significant. CL = confidence limits.

	Mean BCVA (letters) (SD) Range	Mean BCVA (letters)	Age corrected mean difference in BCVA (letters) (95% CL) P value		
		Corrected for age	Diabetic control	Early maculopathy	Sight-threatening maculopathy
Healthy control n=29	90.7 (4.3) 77-97	90.6	-3.8 (-9.6, 2.0) 0.40	-5.8 (-11.3, -0.2) 0.04*	-9.9 (-14.8, -5.1) <0.01*
Diabetic control n=23	86.7 (6.2) 68-95	86.8		-2.0 (-7.8, 3.9) 0.94	-6.2 (-11.5, - 0.9) <0.02*
Early maculopathy n=24	84.8 (6.7) 60-95	84.8			-4.2 (-9.3, 1.0) 0.17
Sight-threatening maculopathy n=41	80.6 (9.8) 55-95	80.6			

Figure 6.2: Boxplot of inter-group comparison of BCVA. Values represented are the median, upper and lower quartiles, and the 95% confidence interval. Outliers are represented by a circle (value more than 1.5 times the length of the box from the end of the box) or an asterisk (value more than 3 times the length of the box from the end of the box). Numbers next to outliers represent the study number of the subject



6.3.2 Contrast sensitivity (Table 6.7, Figure 6.3)

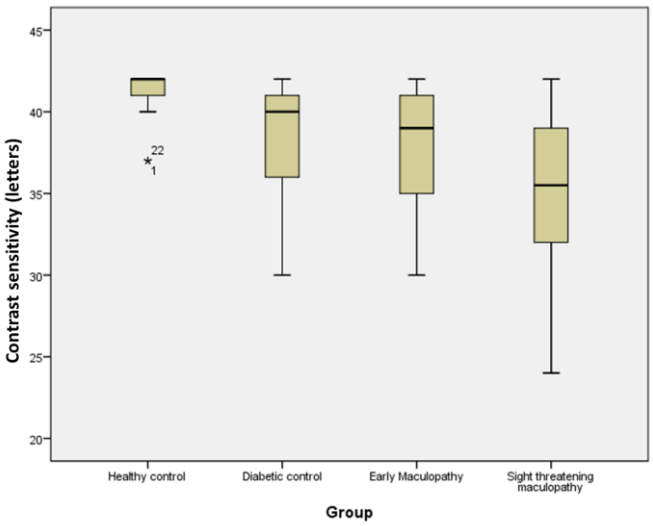
Mean contrast sensitivity (CS) differed significantly across groups (<0.05, ANOVA F-Test). Similar to BCVA, mean CS was significantly reduced in subjects with early maculopathy (38.0 letters, $p<0.05$) and subjects with sight-threatening maculopathy (34.9 letters, $p<0.01$) compared to healthy controls (40.9 letters). There was no significant difference between healthy and diabetic controls (38.7 letters, $p>0.1$).

Subjects with sight threatening maculopathy had a significantly worse mean CS compared to both the diabetic controls ($p<0.01$) and subjects with early maculopathy ($p<0.01$). There was no significant difference between diabetic controls and subjects with early maculopathy ($p>0.50$).

Table 6.7: Intergroup comparison of contrast sensitivity (letters). Mean values corrected for age. P value represents age corrected comparisons. *P<0.05 is deemed significant. CL = confidence limits.

	Mean CS (letters) (SD) Range	Mean CS (letters)	Age corrected mean difference in CS (letters) (95% CI) P value		
		Corrected for age	Diabetic control	Early maculopathy	Sight-threatening maculopathy
Healthy control n=29	41.1 (1.4) 37-42	40.9	-2.2 (-5.1, 0.6) 0.20	-2.9 (-5.6, -0.1) 0.04*	-6.1 (-8.5, -3.6) <0.01*
Diabetic control n=23	38.4 (3.2) 30-42	38.7		-0.6 (-3.5, 2.3) 0.99	-3.8 (-6.4, -1.2) <0.01*
Early maculopathy n=24	38.0 (3.7) 30-42	38.0			-3.2 (-5.7, -0.7) <0.01*
Sight-threatening maculopathy n=40	34.9 (4.9) 24-42	34.9			

Figure 6.3: Boxplot of inter-group comparison of CS. Values represented are the median, upper and lower quartiles, and the 95% confidence interval. Outliers are represented by a circle (value more than 1.5 times the length of the box from the end of the box) or an asterisk (value more than 3 times the length of the box from the end of the box). Numbers next to outliers represent the study number of the subject



6.3.3 Microperimetry (Table 6.8, Figure 6.4)

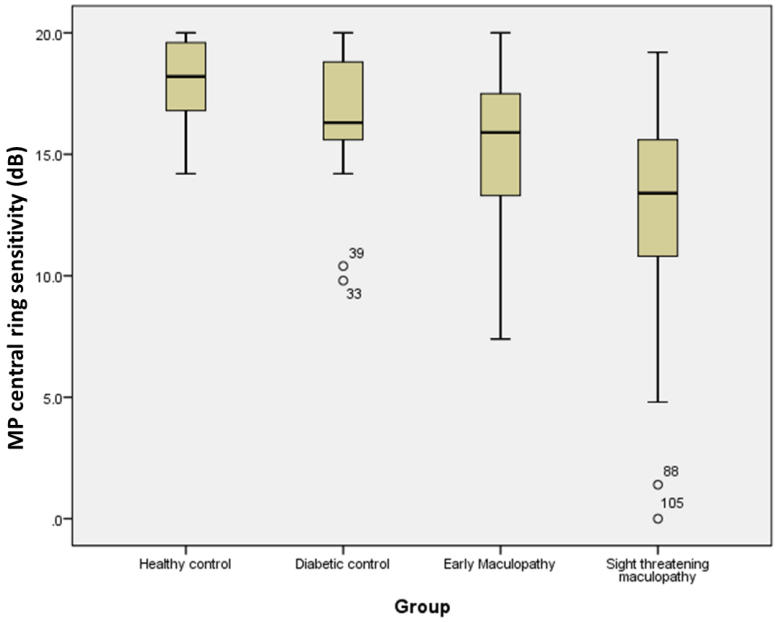
Mean microperimetry (MP) central ring sensitivities differed significantly between groups ($p<0.01$, ANOVA F-Test) and are shown in Table 6.8. Mean central ring sensitivity was significantly reduced in subjects with sight-threatening maculopathy (12.7 dB) compared to healthy controls (17.9 dB, $p<0.01$). Subjects with early maculopathy showed a trend towards reduced mean sensitivity (15.2 dB, $p>0.05$). There was no significant difference between healthy controls and diabetic controls (16.5 dB, $p>0.50$).

Subjects with sight threatening maculopathy had a significantly reduced mean sensitivity compared to diabetic controls ($p<0.01$) and there was a trend towards reduced mean sensitivity compared to subjects with early maculopathy ($p<0.05$). There was no significant difference between diabetic controls and subjects with early maculopathy ($p>0.50$).

Table 6.8: Intergroup comparison of MP central ring sensitivity without and with correction for age. P value represents age corrected comparison. *P ≤0.01 deemed significant. CL = confidence limits.

	Mean MP sensitivity (dB) (SD) Range	Mean MP sensitivity (dB)	Age corrected mean difference in MP sensitivity (dB) (95% CL) P value		
		Corrected for age	Diabetic control	Early maculopathy	Sight-threatening maculopathy
Healthy control n=25	17.9 (1.9) 14.2-20.0	17.9	-1.4 (-4.4, 1.7) 0.79	-2.7 (-5.5,0.1) 0.06	-5.2 (-7.7,-2.8) <0.01*
Diabetic control n=18	16.4 (2.8) 9.8-20.0	16.5		-1.3 (-4.3,1.7) 0.82	-3.8 (-6.6,-1.1) <0.01*
Early maculopathy n=24	15.2 (3.3) 7.4-20.0	15.2			-2.5 (-5.0,-0.1) 0.04
Sight-threatening maculopathy n=41	12.7 (4.6) 1.4-19.2	12.7			

Figure 6.4: Boxplot of inter-group comparison of MP. Values represented are the median, upper and lower quartiles, and the 95% confidence interval. Outliers are represented by a circle (value more than 1.5 times the length of the box from the end of the box) or an asterisk (value more than 3 times the length of the box from the end of the box). Numbers next to outliers represent the study number of the subject



6.3.4 Multifocal electroretinogram (Tables 6.9 and 6.10, Figure 6.5)

The mean mfERG central ring amplitudes are shown in Table 6.9. Mean mfERG central ring amplitude significantly differed across groups ($p<0.01$, ANOVA F-test). Mean amplitude was significantly reduced in subjects with sight-threatening maculopathy (41.7 nV/deg^2) as compared to healthy controls (76.0 nV/deg^2, $p<0.01$). There was a trend towards reduced mean amplitude in subjects with early maculopathy (58.6 nV/deg^2, $p<0.05$). There was no significant difference compared to diabetic controls (75.6 nV/deg^2, $p>0.50$).

Subjects with sight threatening maculopathy showed reduced mean amplitude compared to diabetic controls ($p<0.01$) and a trend towards reduced amplitude compared to subjects with early maculopathy ($p<0.05$). There was also a trend towards reduced mean amplitude in subjects with early maculopathy compared to diabetic controls ($p>0.05$).

The mean central ring mfERG implicit times (IT) are shown in Table 6.10. Mean IT differed across groups ($p<0.01$, ANOVA F-test) and was significantly prolonged in subjects with sight-threatening maculopathy (40.3 ms) compared to healthy controls (37.8 ms, $p<0.01$). Though mean IT was prolonged in subjects with early maculopathy, this did not reach statistical significance (38.9 ms, $p>0.50$). There was no difference between healthy controls and diabetic controls (37.7 ms $p>0.50$).

Mean IT was significantly prolonged in sight-threatening maculopathy compared to diabetic controls ($p\leq0.01$). It was also prolonged in sight threatening maculopathy compared to early maculopathy though this did not reach statistical significance ($p>0.10$). There was no significant difference between diabetic controls and subjects with early maculopathy ($p>0.50$).

Figure 6.5: Boxplot of inter-group comparison of mfERG central ring amplitude and implicit time. Values represented are the median, upper and lower quartiles, and the 95% confidence interval. Outliers are represented by a circle (value more than 1.5 times the length of the box from the end of the box) or an asterisk (value more than 3 times the length of the box from the end of the box). Numbers next to outliers represent the study number of the subject

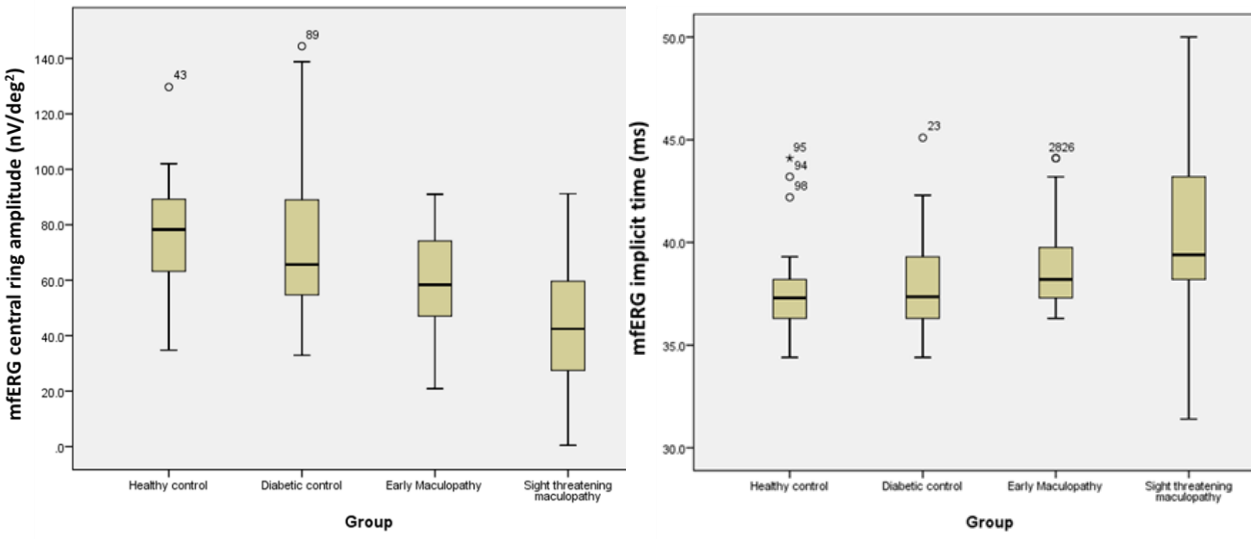


Table 6.9. Intergroup comparison of mean mfERG central ring amplitude without and with correction for age. P value represents age corrected comparisons. P<0.01 is deemed significant* CL = confidence limits.

	Mean mfERG amplitude (nV/deg^2) (SD) Range	Mean mfERG amplitude (nV/deg^2)	Age corrected mean difference in mfERG amplitude (nV/deg^2) (95% CL) P value		
		Corrected for age	Diabetic control	Early maculopathy	Sight-threatening maculopathy
Healthy control n=29	76.4 (20.1) 44.5-129.7	76.0	-0.3 (-18.5, 17.8) 1.00	-17.4 (-34.7, -0.1) <0.05	-34.4 (-49.6, -19.1) <0.01*
Diabetic control n=22	75.0 (32.0) 32.9-144.4	75.6		-17.1 (-35.5, 1.4) 0.09	-34.0 (-50.7, -17.3) <0.01*
Early maculopathy n=24	58.4 (18.4) 20.9-91.0	58.6			-17.0 (-33.0, -0.9) 0.03
Sight-threatening maculopathy n=40	41.7 (21.9) 0.5-91.2	41.6			

Table 6.10: Intergroup comparison of mfERG central ring implicit time without and with correction for age. P value represents age corrected comparison. *P<0.01 is deemed significant. CL = confidence limits.

	Mean mfERG implicit time (ms) (SD) Range	Mean mfERG implicit time (ms)	Age corrected mean difference in mfERG implicit time (ms) (95% CL) P value		
		Corrected for age	Diabetic control	Early maculopathy	Sight-threatening maculopathy
Healthy control n=29	37.6 (2.3) 35.3-44.1	37.8	-0.1 (-2.6, 2.3) 1.00	0.9 (-1.4, 3.3) 0.87	2.5 (0.4, 4.6) <0.01*
Diabetic control n=22	38.1 (2.8) 34.4-45.1	37.7		1.1 (-1.4 to 3.6) 0.83	2.6 (0.4, 4.9) 0.01
Early maculopathy n=24	38.9 (2.4) 36.3-44.1	38.8			1.6 (-0.6, 3.7) 0.30
Sight-threatening maculopathy n=40	40.3 (4.3) 31.4-50.0	40.3			

6.3.5 Oscillatory potentials (Tables 6.11 and 6.12, Figure 6.6)

Mean OP sum amplitudes are shown in Table 6.11 and differed significantly across groups ($p<0.01$, ANOVA F-test). Mean OP sum amplitude was significantly reduced in subjects with sight threatening maculopathy (44.9 µV) as compared to healthy controls (69.5 µV, $p<0.01$), diabetic controls (68.7 µV, $p\leq0.01$) and subjects with early maculopathy (74.5 µV, $p<0.01$). There were no significant differences between any of the other groups ($p>0.50$ for all comparisons).

Mean OP 1st peak implicit times are shown in Table 5.12. OP implicit time differed significantly across groups ($p<0.01$, ANOVA F-test). Mean OP IT showed a trend towards prolongation in sight-threatening maculopathy (18.8ms) compared to healthy controls (17.7 ms, $p\leq0.02$). Mean OP IT in diabetic controls (18.6 ms, $p>0.10$) and early maculopathy (18.0 ms, $p>0.50$) was non-significantly prolonged compared to healthy controls. Early maculopathy showed a shorter mean OP IT compared to the other groups with DM but this was statistically not significant ($p>0.20$ for all comparisons).

Figure 6.6: Boxplot of inter-group comparison of OP sum amplitude and OP 1st peak implicit time. Values represented are the median, upper and lower quartiles, and the 95% confidence interval. Outliers are represented by a circle (value more than 1.5 times the length of the box from the end of the box) or an asterisk (value more than 3 times the length of the box from the end of the box). Numbers next to outliers represent the study number of the subject

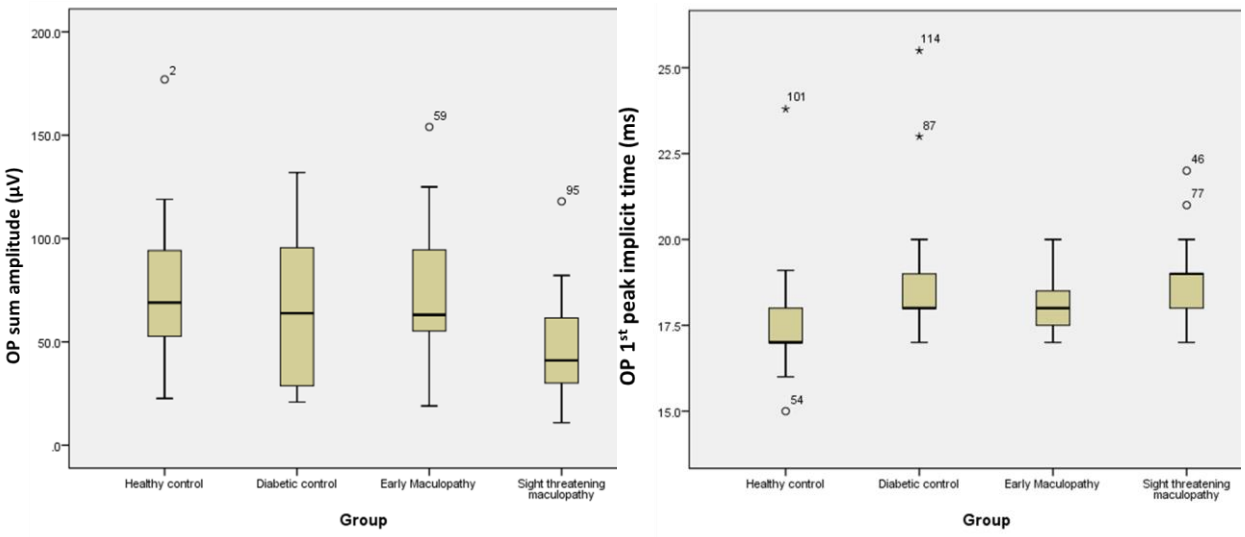


Table 6.11: Intergroup comparison of mean OP sum amplitude without and with correction for age. P value represents age corrected. *P<0.01 is deemed significant. CL = confidence limits.

	Mean OP sum amplitude (μV) (SD) Range	Mean OP sum amplitude (μV)	Age corrected mean difference in OP sum amplitude (μV) (95% CL) P value		
		Corrected for age	Diabetic control	Early maculopathy	Sight-threatening maculopathy
Healthy control n=29	73.3 (32.5) 22.6-177.0	69.5	-0.8 (-22.2, 20.7) 1.00	5.0 (-15.5,25.4) 0.99	-24.5 (-42.6, -6.5) <0.01*
Diabetic control n=22	63.4 (34.5) 23.2-132.0	68.7		5.7 (-16.1,27.5) 0.98	-23.8 (-43.7, -3.9) 0.01
Early maculopathy n=24	73.3 (31.5) 19.0-154.0	74.5			-29.5 (-48.6, -10.4) <0.01*
Sight-threatening maculopathy n=39	45.8 (22.9) 12.7-118.0	44.9			

Table 6.12: Intergroup comparison of OP 1st peak implicit time without and with correction for age. p value represents comparison to diabetic controls, early maculopathy, sight-threatening maculopathy. *P<0.01 is deemed significant. CL = confidence limits

	Mean OP implicit time (ms) (SD) Range	Mean OP implicit time (ms)	Age corrected mean difference in OP implicit time (ms) (95% CL) P value		
		Corrected for age	Diabetic control	Early maculopathy	Sight-threatening maculopathy
Healthy control n=29	17.6 (1.5) 15.0-23.8	17.7	1.0 (-0.2,1.9) 0.18	0.3 (-0.7,1.3) 0.96	1.0 (0.1,1.9) 0.02
Diabetic control n=21	18.7 (2.1) 17.0-25.5	18.6		-0.5 (-1.6,0.5) 0.70	0.2 (-0.8,1.2) 1.00
Early maculopathy n=24	18.1 (0.9) 17.0-20.0	18.0			0.7 (-0.2,1.6) 0.25
Sight-threatening maculopathy n=38	18.7 (1.1) 17.0-22.0	18.8			

6.3.6 Optical coherence tomography (Table 6.13)

Mean OCT central subfield thicknesses (CSFT) are shown in Table 6.13. Mean OCT CSFT differed significantly across groups ($p<0.01$, ANOVA F-test).There were no significant differences in mean CSFT between healthy controls, diabetic controls and early maculopathy ($p>0.50$ for all comparisons). Mean CSFT was significantly thicker in sight-threatening maculopathy compared to all other groups (>60 µm, $p<0.01$ for all comparisons).

6.3.7 Ischaemic maculopathy (Table 6.14)

Sub-analysis of the sight-threatening maculopathy group is presented in Table 6.14. Subjects with ischaemic macular changes showed a significantly reduced mean BCVA, contrast sensitivity, MP central ring sensitivity and mfERG central ring amplitude compared to those with no ischaemic changes ($p<0.01$ for all comparisons). There was a reduced mean OP sum amplitude in subjects with ischaemic macular changes but this was not statistically significant ($p>0.50$). Implicit time for both mfERG central ring and OP 1st peak were prolonged in those with ischaemic maculopathy but this again did not reach statistical significance ($p>0.50$).

Subjects with ischaemic macular changes showed prolongation of mean mfERG central ring IT compared to both healthy (4.2 ms, $p<0.01$) and diabetic controls (4.3 ms, $p<0.01$). Mean OP IT was also prolonged compared to healthy controls though this did not reach statistical significance (1.5 ms, $p<0.10$).

After exclusion of subjects with ischaemic maculopathy, subjects with only CSMO still showed reduced retinal function compared to healthy controls in BCVA (-7.2 letters, $p<0.01$), CS (-4.7 letters, $p<0.01$), MP central ring sensitivity (-3.6 dB, $p<0.01$), and mfERG central ring amplitude (-27.4 nV/deg^2, $p<0.01$). There was a trend towards reduced OP sum amplitude (-20.7 µV, $p<0.05$). Mean mfERG central ring IT was no longer significantly prolonged (2.0ms, $p>0.10$).

Table 6.13: Intergroup comparison of OCT central subfield thickness (CSFT) without and with correction for age. P value represents age corrected comparison. *P<0.05 is deemed significant. CL = confidence limits

	Mean OCT CSFT (µm) (SD) Range	Mean OCT CSFT (µm) (SD) Range	Age corrected mean OCT CSFT (µm) (95% CL) P value		
		Corrected for age	Diabetic control	Early maculopathy	Sight-threatening maculopathy
Healthy control n=28	283.7 (19.0) 235-340	281.6	-9.1 (-52.3, 34.1) 1.00	-2.8 (-44.1, 38.5) 1.00	60.5 (24.1, 96.9) <0.01*
Diabetic control n=22	269.6 (22.8) 211-306	272.4		6.3 (-37.3, 49.9) 1.00	69.6 (30.0, 109.2) <0.01*
Early maculopathy n=24	278.2 (31.1) 190-349	278.8			63.3 (25.3, 101.3) <0.01*
Sight-threatening maculopathy n=40	342.5 (86.0) 242-636	342.1			

Table 6.14: Sub-analysis of sight-threatening maculopathy group, comparing subjects with ischaemic maculopathy to subjects CSMO only. Mean values corrected for age. *P<0.01 is deemed significant

	CSMO (n=32)	Ischaemic maculopathy (n=9)	P value
Mean BCVA (letters) (SD)	83.4 (7.1)	70.8 (12.0)	<0.01*
Mean CS (letters) (SD)	36.2 (4.2)	30.3 (4.7)	<0.01*
Mean MP central ring sensitivity (dB) (SD)	14.3 (3.1)	6.9 (4.6)	<0.01*
Mean mfERG central ring amplitude (nV/deg^2) (SD)	48.5 (19.1)	17.7 (11.8)	<0.01*
Mean mfERG central ring implicit time (ms) (SD)	39.8 (3.8)	42.0 (5.8)	0.50
Mean OP sum amplitude (μV) (SD)	48.8 (21.7)	32.2 (23.2)	0.69
Mean OP implicit time (ms) (SD)	18.0 (1.1)	18.6 (1.0)	0.94

6.4 Analysis of association and of diagnostic potential

I analysed the outcomes of visual function to determine the probability that a subject with DM and compromised function has sight-threatening maculopathy. As described in Chapter 4, Section 7.3, compromised function of an investigation was determined by the quartile closest to reduced function and what I have termed the critical value. This was deemed to be the lower quartile for BCVA, CS, MP, mfERG amplitude and OP amplitude, and the upper quartile for mfERG and OP implicit times. The Fisher exact test of association was used to determine significance;

$p<0.05$ was deemed significant. Binomial confidence intervals were calculated using Clopper-Pearson formula.

In addition to association, I also calculated the sensitivity, specificity, positive predictive value and negative predictive value for each test.

- Sensitivity: proportion of subjects with sight-threatening maculopathy with function equal to or worse than the critical value
- Specificity: proportion of subjects without sight-threatening maculopathy with function better than the critical value
- Positive predictive value (PPV): proportion of subjects with function equal to or worse than critical value who have sight-threatening maculopathy
- Negative predictive value (NPV): proportion of subjects with function better than critical value who do not have sight-threatening maculopathy

6.4.1 Analysis of single functional outcomes (Table 6.15)

In Table 6.15, I present the results of using one outcome in analysing likelihood of a subject having sight-threatening maculopathy. The association tests the likelihood that a subject with visual or retinal function worse than or equal to the critical value has sight-threatening maculopathy.

There were significant associations between the presence of sight-threatening maculopathy and function worse than the critical value for BCVA ($p<0.01$), CS ($p<0.001$), MP ($p<0.03$), mfERG amplitude ($p<0.01$) and OP IT ($p<0.02$). For all investigations sensitivity was low (33.3 - 55.3%) but specificity was high (73.3 – 89.4%). Each test showed moderate PPV (61.9 – 78.3%) and NPV (58.1 – 66.0 %).

Table 6.15: Univariate associations of central macular function and sight-threatening maculopathy with analysis of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). amp = amplitude; IT = implicit time. *P < 0.05 is deemed significant. CI = confidence interval

	Critical value	Association (p value)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV(%) (95% CI)	NPV(%) (95% CI)
BCVA (letters)	≤79.0	<0.01*	41.5 (26.3-57.9)	85.1 (71.7-93.8)	70.8 (48.9-87.4)	62.5 49.5-74.3
CS (letters)	≤34.0	<0.001*	45.0 (29.3-61.5)	89.4 (76.9-96.5)	78.3 (56.3-92.5)	65.6 (52.7-77.1)
MP (dB)	≤12.6	<0.03*	36.6 (22.1-53.1)	85.7 (71.5-94.6)	71.4 (47.8-88.7)	58.1 (44.9-70.5)
mfERG amp (nV/deg^2)	≤33.9	<0.01*	40.0 (24.9-56.7)	89.1 (76.4-96.4)	76.2 (52.8-91.8)	63.1 (50.2-74.7)
mfERG IT (ms)	≥41.2	>0.05	37.5 (22.7-54.2)	82.6 (68.6-92.2)	65.2 (42.7-83.6)	60.3 (47.2-72.4)
OP sum amp (µV)	≤34.5	>0.10	33.3 (19.1-50.2)	82.6 (68.6-92.2)	61.9 (38.4-81.9)	59.4 (46.4-71.5)
OP IT (ms)	≥19.0	<0.02*	55.3 (38.3-71.4)	73.3 (58.1-85.4)	63.6 (45.1-79.6)	66.0 (51.2-78.8)

6.4.2 Analysis of combined functional outcomes (Table 6.16 and Table 6.17)

In Table 6.16, I present the results of association of visual function to sight-threatening maculopathy if two investigations are combined, i.e a subject has reduced visual function in two separate investigations. In Table 6.17 I present the results of association of central macular function to sight-threatening maculopathy.

By combining two investigations, there is a significant association to sight-threatening maculopathy in all investigations except for the following combinations: MP + mfERG IT; mfERG IT + OP amplitude; mfERG + OP IT; OP amplitude + OP IT.

Sensitivity was low for all comparisons (15.4 – 30.0%) but specificity was high (87.0 – 100.0%). For combinations with significant association to sight-threatening maculopathy, PPV was high (83.3 – 100.0%) and NPV was moderate (56.4 – 62.0%).

Table 5.16: Bivariate associations of visual function and sight-threatening maculopathy. amp = amplitude; IT = implicit time. *P < 0.05 is deemed significant.

Investigation	Association (p value)	Sensitivity(%) (95% CI)	Specificity(%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
BCVA + CS	<0.01*	30.0 (16.6-46.5)	93.6 (82.5-98.7)	80.0 (51.9-95.7)	61.1 (48.9-72.4)
BCVA + MP	<0.01*	29.3 (161.-45.5)	100.0 (91.2-100.0)	100.0 (73.5-100.0)	58.0 (45.5-69.8)
BCVA + mfERG amp	<0.01*	25.0 (12.7-41.2)	100.0 (92.1-100.0)	100.0 (69.2-100.0)	60.0 (48.0-71.2)
BCVA + mfERG IT	<0.03*	17.1 (7.2-32.1)	97.8 (88.2-99.9)	87.5 (47.4-99.7)	56.4 (44.7-67.6)
BCVA + OP amp	<0.05*	15.4 (5.9-30.5)	97.8 (88.2-99.9)	85.7 (42.1-99.6)	57.1 (45.5-68.4)
BCVA + OP IT	<0.01*	23.7 (11.4-40.2)	97.7 (88.0-99.9)	90.0 (55.5-99.8)	59.7 (47.5-71.1)
CS+MP	<0.01*	27.5 (14.6-43.9)	97.6 (87.1-99.9)	91.7 (61.5-99.8)	58.0 (45.5-69.8)
CS + mfERG amp	<0.01*	25.6 (13.0-42.1)	97.8 (88.2-99.9)	90.9 (58.7-99.8)	60.3 (48.1-71.6)
CS + mfERG IT	<0.01*	17.5 (7.3-32.8)	100.0 (92.1-100.0)	100.0 (59.0-100.0)	57.7 (46.0-68.8)
CS + OP amp	<0.01*	20.5 (9.3-36.5)	100.0 (92.1-100.0)	100.0 (63.1-100.0)	59.2 (47.3-70.4)
CS + OP IT	<0.01*	30.8 (17.0-47.6)	100.0 (92.0-100.0)	100.0 (73.5-100.0)	62.0 (49.7-73.2)

Table 6.17: Bivariate associations and diagnostic potential of central macular function in sight-threatening maculopathy when two outcomes are combined. amp = amplitude; IT=implicit time. *P<0.05 is deemed significant. CI=confidence interval

Investigation	Association (p value)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
MP + mfERG amp	<0.02*	25.0 (12.7-41.2)	95.2 (83.8-99.4)	83.3 (51.6-97.9)	57.1 (44.8-68.9)
MP + mfERG IT	>0.10	17.5 (7.3-32.8)	92.9 (80.5-98.5)	70.0 (34.8-93.3)	54.2 (42.0-66.0)
MP + OP amp	<0.01*	17.9 (7.5-33.5)	100.0 (91.6-100.0)	100.0 (59.0-100.0)	56.8 (44.7-68.2)
MP + OP IT	<0.01*	25.6 (13.0-42.1)	97.6 (87.4-99.9)	90.9 (58.7-99.8)	58.6 (46.4-70.2)
mfERG amp + mfERG IT	<0.01*	17.5 (7.3-32.8)	100.0 (92.3-100.0)	100.0 (59.0-100.0)	58.2 (46.6-69.2)
mfERG amp + OP amp	<0.03*	18.4 (7.7-34.3)	97.8 (88.5-99.9)	87.5 (47.4-99.8)	59.2 (47.3-70.3)
mfERG amp + OP IT	<0.01*	26.3 (13.4-43.1)	97.8 (88.2-99.9)	90.9 (58.7-99.8)	61.1 (48.9-72.4)
mfERG IT + OP amp	>0.10	17.9 (7.5-33.5)	93.5 (82.1-98.6)	70.0 (34.8-93.3)	57.3 (45.4-68.7)
mfERG IT + OP IT	>0.10	28.2 (15.0-44.9)	87.0 (73.7-95.1)	64.7 (38.3-85.8)	58.8 (46.2-70.6)
OP amp + OP IT	>0.05	28.9 (15.4-45.9)	88.9 (75.9-96.3)	68.8 (41.3-89.0)	59.7 (47.0-71.5)

6.5 Discussion

6.5.1 Key results

In this chapter I have found the following absolute statistically significant worsening in mean visual function corrected for age for sight threatening maculopathy:

- a two line (9.9 letters) reduction in BCVA compared to healthy controls and just over one line (6.2 letters) reduction compared to diabetic controls
- 15% (6.1 letters) reduction in contrast sensitivity compared to healthy controls, 10% (3.8 letters) reduction compared to diabetic controls and 8% (3.2 letters) compared to early maculopathy
- 29% reduction in microperimetry sensitivity compared to healthy controls and 23% reduction compared to diabetic controls
- 45% reduction in mfERG central ring amplitude compared to both healthy controls and diabetic controls
- 7% prolongation of mfERG central ring implicit compared to both healthy and diabetic controls
- 35% reduction in OP sum amplitude compared to both healthy and diabetic controls and 40% reduction compared to early maculopathy
- trend towards prolongation of OP 1st peak implicit time

In sight-threatening maculopathy this is followed by the following significant changes in structure:

- an increase in 20-25% in mean CSFT on OCT

For early maculopathy there were trend associations, all indicating reduced function:

- reduction in BCVA of over one line (5.8 letters) compared to healthy controls
- reduction in CS (2.9 letters) compared to healthy controls
- 15% reduction in MP central ring sensitivity compared to healthy controls
- 23% reduction in mfERG amplitude compared to healthy and diabetic controls

For diabetic controls compared to healthy controls there was reduced function across many measures but none of the differences reached statistical significance.

6.5.2 Microperimetry

In my study, subjects with sight-threatening maculopathy (12.7 dB) showed a 29% reduction in central ring sensitivity compared to healthy controls (17.9 dB, $p<0.01$) and a 23% reduction compared to diabetic controls (16.5 dB, $p<0.01$). There was a trend towards reduced function compared to subjects with early maculopathy (15.2 dB, $p=0.04$). Subjects with early maculopathy demonstrated a 15% reduction in sensitivity compared to healthy controls though this difference did not reach significance ($p=0.06$).

My findings are similar to a study by Okada et al (2006).[183] The authors retrospectively assessed MP sensitivity in the central 2^o of the macula in subjects with CSMO without ischaemic macular changes (n=32) and compared them to healthy controls (n=17). The authors reported significantly reduced sensitivity ($p<0.0001$) in subjects with CSMO and correlated sensitivity to BCVA ($r^2=0.62$, $p<0.0001$) and CSFT ($r^2=0.58$, $p<0.0001$). The authors concluded that MP may be used as a measure to assess the effects of CSMO. However this was a retrospective analysis with no correction for risk factors or age. Median CSFT was 523 µm and median BCVA was ~6/30, while my subjects had better BCVA and less foveal thickening. It is, therefore, possible that subjects in this study had more advanced disease.

Other studies have also reported reduced function in subjects with CSMO.[184,185] However, these studies assessed the macula as a whole rather than just the central macula. Al-Shafaee et al (2011)[184] demonstrated reduced function in all subjects with DMO, though the authors did not report whether these subjects had CSMO or early maculopathy. Gella et al (2016)[185] prospectively analysed retinal sensitivity in 357 subjects with type 2 DM. The authors only reported reduced sensitivity at 8^o, 10^o and 12^o in subjects with DMO, but not in the central 2^o or 4^o. However the authors did not report whether these subjects had CSMO. The authors concluded

that the presence of macular oedema was associated with significant reduction in function.

In my study, subjects with CSMO showed reduced function compared to subjects with early maculopathy though this was not statistically significant (p=0.04). There have been no studies which have compared between different severities of diabetic maculopathy. There has been a study comparing different severities of DR. Nittala et al (2012)[186] assessed retinal sensitivity between healthy controls, diabetic controls, subjects with mild, moderate and severe non-proliferative DR (NPDR) and subjects with proliferative DR (PDR). The authors reported a reduction in sensitivity in subjects with DR (p=0.001). Sub-analysis reported the following mean sensitivities in the central 2^{o} of the macula: healthy controls, 16.68 dB; diabetic controls, 14.73 dB; mild NPDR, 14.87 dB; moderate NPDR, 13.37 dB; severe NPDR, 8.46 dB; PDR, 8.6 dB. However p values were not reported for comparison between the NPDR groups. Nevertheless, there appears to be a dramatic decrease in MP sensitivity in subjects who develop severe disease. This may indicate a critical point in DR after which function decreases more rapidly.

The presence of ischaemic macular changes resulted in a 61% reduction in retinal sensitivity compared to healthy controls in my study. This finding is similar to results published by Cennamo et al (2015)[187] who reported a 59% reduction in sensitivity in subjects with ischaemic maculopathy as compared to healthy controls. These subjects showed a significant reduction in retinal thickness compared to retinal thickness, whilst our subjects had clinically significant macular oedema (CSMO) in all but one case and so had an overall increased thickness compared to healthy controls.

In my study, comparison between subjects with CSMO and ischaemic maculopathy revealed a significant reduction in sensitivity of 51% in the latter group. This suggests that the presence of macular ischaemia is a risk factor for reduced central macular function. Therefore the clinician should be suspicious of the presence of ischaemic macular changes in subjects with CSMO and severely reduced central retinal sensitivity.

In my study, there was no significant difference between healthy controls and diabetic controls, though sensitivity was reduced on average by 8%. De Benedetto et al (2014) reported a less than 10% reduction in sensitivity between subjects with non-proliferative DR and healthy controls.[188]

Other studies have also reported reduced sensitivity in subjects with no DR compared to healthy controls. However these studies have either used the whole macula for analysis,[31,189] did not report p values,[184] used only 1 point for assessment of central macular function,[186] or included subjects with varying levels of retinopathy within the main analysis.[190] In my study I analysed only the central macula using an average of five points to provide a more accurate reflection of foveal function.

Nittala et al (2012) reported no significant difference between subjects with no DR and those with mild to moderate DR.[186] Al Shafaee et al (2011)[184] reported 24% of healthy controls and 20% of diabetic controls as having abnormal function. However the authors have not reported what constitutes abnormal function. Gella et al (2016) reported no significant difference between subjects with no DR and those with macular oedema in the central ring of the OCT.[185] This suggests that decrease in function occurs in a step-wise fashion rather than a linear pattern, i.e. there is an initial dysfunction which is preserved until severe disease develops. These results are similar to my study in which function continues to decrease with increasing severity across maculopathy groups though statistical significance was only reached in the sight-threatening maculopathy group. It is possible that larger groups may have provided significant results in the inter-group comparisons in earlier disease groups.

Other features in diabetic maculopathy have been associated with reduced sensitivity. In a study of subjects with DMO, point sensitivity was analysed in the presence of disruption of the photoreceptor layer.[191] The authors reported a mean 3.28 dB reduction in retinal sensitivity at points with disruption compared to points without disruption. However there was a significant increase in retinal thickness in subjects at points with disruption compared to those without disruption. Therefore

the difference in sensitivity may reflect difference in retinal thickness rather than photoreceptor layer disruption. In a study of 12 eyes with exudates, mean retinal sensitivity over regions with exudates was associated with reduced retinal sensitivity compared to regions without exudates.[192] However, retinal thickness was significantly increased in the regions and not adjusted for in analysis. In a study of 34 eyes with DMO, retinal sensitivity was compared to type of DMO.[193] The authors reported a significant difference between subjects with diffuse retinal thickening, cystoid macular oedema (CMO), subretinal fluid (SRF) and neurosensory retinal detachment (NRD). The authors concluded that diabetic macular oedema progressed from diffuse thickening to CMO, with the latter associated with worse retinal function, and subsequently progressed to the more severe forms of SRF and NRD. I did not assess for these potential risk factors but these studies suggest the presence of such features should alert the clinician to probable reduced function.

6.5.3 Multifocal electroretinogram

Subjects with sight threatening maculopathy demonstrated significant reduction in amplitude (41.6 nV/deg^2) and prolongation of IT (40.3 ms) compared to healthy controls (76.0 nV/deg^2 and 37.8 ms, respectively; $p<0.01$ for both comparisons) and diabetic controls (75.6 nV/deg2 and 37.7 ms, respectively; $p<0.01$ and $p=0.01$, respectively). Subjects with sight-threatening maculopathy showed a trend towards reduced amplitude compared to early maculopathy (58.6 nV/deg^2, $p<0.03$) and a non-significant prolongation of IT (38.8 ms, $p>0.20$).

Similar findings have been reported in several studies. Yamamoto et al (2001) reported a >50% decrease in amplitude and prolongation in IT of ~15% in the presence of DMO compared to healthy controls.[194] This study analysed the central 10^o of the macula, a larger area than I have analysed, and did not perform fluorescein angiography (FA) to assess for ischaemic maculopathy. Tehrani et al (2015) reported compared mfERG amplitude and IT in 29 subjects with CSMO to healthy controls.[195] The authors reported reduced amplitude and prolonged IT in subjects with CSMO in the central macula (5^o).

Greenstein et al (2000) reported similar findings with respect to amplitude and IT in DMO.[152] Using a 103 hexagon array, the authors reported that IT appeared to be more severely affected than amplitude. However the authors reported findings of the whole macula rather than central macula only. The authors concluded that though function is reduced in CSMO, IT was not a good predictor of reduced function due to wide variation in function in disease.

It is important to note that subjects with early and sight-threatening maculopathy contained subjects with normal amplitude and IT. This reflects the variable structural changes in the macula in subjects with DMO. Hood suggests that damage to different structures may result in varying mfERG results.[153] He surmised that disruption of the ganglion cell layer may not alter amplitude or IT; disruption of the cone receptors, outer plexiform layer or on-bipolar cells may result in decreased amplitude and IT; disruption of the inner plexiform layer may only affect IT; and disruption of the off-bipolar cells may actually be associated with improved function. This suggests that combined assessment of structure and function would yield more information on severity of disease.

In my study subjects with early maculopathy showed a trend towards reduction in central ring amplitude compared to healthy controls ($p<0.05$) and a non-significant reduction in amplitude compared to diabetic controls ($p>0.05$). Implicit time (IT) was non-significantly prolonged compared to both healthy and diabetic controls ($p>0.50$ for both comparisons).

Holm et al (2010) reported that the presence of exudates was associated with prolonged IT.[148] Amplitude was reduced but only in the parafoveal and extrafoveal regions. The difference between amplitude and IT results between my study and published literature is most likely related to how the mfERG stimulus is set up. I will discuss this further in the summary of mfERG findings below.

In my study there was no significant difference in amplitude or IT between diabetic and healthy controls. This replicates findings of Wright et al (2012)[151] who reported no significant difference in the central ring in subjects with type 1 DM, and Dhamdhere et al (2012)[196] who reported no significant reduction in function in

subjects with type 1 DM. However several studies have reported significant changes in amplitude, IT or both in subjects with no DR.

Reis et al (2014) reported a significant reduction in mfERG amplitude only in the central macula.[197] However this study only included type 1 DM subjects with a mean duration of DM of >14 years which is much longer than my cohort. Tyrberg et al (2005) also reported decreased amplitude only, though their subjects had a mean duration of DM >23 years.[198] Lung et al (2012) also reported reduced amplitude but no change in IT.[199]

Several studies have reported mfERG IT to be more sensitive with prolongation in IT seen but no significant difference in amplitude.[150,200-202] The reason IT may be more sensitive is that amplitude reflects the strength of summed responses from retinal cells.[201] Therefore amplitude is likely to be affected only by loss of cells. IT on the other hand reflects efficiency of retinal cell function. Therefore, in disease, there may be disruption of cell function without loss of the cell and results seen reflect inefficiency rather than permanent structural change.

A few studies have reported both decrease in amplitude and prolongation of IT.[203] Bronson-Castain et al (2012) and Dhamdhere et al (2012) reported significant changes in type 2 DM but not type 1 DM, though there were only 10 patients in type 1 cohort.[196,204]

The difference between my findings and those of the studies listed above may be due to how the mfERG is performed. In my study we used a 19 hexagon grid; the studies described above use either a 61 or 103 hexagon grid. Therefore each hexagon in my study is larger than those used in the other grids. Although this allows for quicker data acquisition, the larger stimulus may contain proportionally healthier rather than diseased retina.[205] This may explain why there was no significant difference between the early maculopathy group and diabetic controls for both amplitude and implicit time. In addition our mfERG system flashes stimuli at a rate of 60 Hz whilst the other studies have a flash rate of 75Hz. With the incorporation of 4 blank filler frames for every stimulus, this results in a stimulus being presented every 83.3 ms; other studies presented a stimulus every 16.6 ms.[137]

This stimulus was chosen as it allows the neurons time to repolarise prior to the next stimulus. (*Richard Hagan, personal communication*) By presenting a stimulus every 16.6 ms, neurons may not have enough time to repolarise. So a compound effect may result in which any minimal delay is magnified by subsequent stimuli to give a more significant delay at the end of the procedure. By allowing repolarisation, any delay in IT would reflect true dysfunction of the stimulated neurons. This may explain why there was no significant difference between healthy and diabetic controls as there may not be a significant number of neurons affected.

Two studies have stated that the nasal macula appears to be more susceptible to damage in early disease.[202,206] Therefore our analysis of just the central macula may have missed neuronal dysfunction in early disease.

Central ring amplitude was significantly worse in subjects with ischaemic maculopathy (17.7 nV/deg^2) compared to subjects with CSMO (48.5 nV/deg^2, $p<0.01$). Though IT was prolonged in the ischaemic group (42.0ms vs 39.8 ms, $p>0.50$) this was not statistically significant.

Tyrberg et al (2008)[207] reported that an enlarged foveal avascular zone (mean diameter 0.92 mm) was associated with prolonged IT in the central ring. A decrease in amplitude was noted but no correlation to ischaemia was found. The difference in mfERG set up between my study and Tyrberg et al (2008) may once again explain the difference in outcomes between the two studies.

In summary, mfERG reveals decreased function in subjects with DM. It has the potential to identify subjects with neuronal dysfunction in early disease. I feel mfERG offers a method of determining disease severity and, in conjunction with other methods, could aid in building a comprehensive picture of a subjects disease status. However the outcomes generated may differ depending on parameters used and so my results may not be applicable to different cohorts. Use of an approved protocol would help to standardise the results and improve our understanding of diabetic maculopathy.

6.5.4 Oscillatory potentials

Mean OP sum amplitude (44.9 µV) was significantly reduced in the sight-threatening maculopathy group compared to healthy controls (69.5 µV, $p<0.01$), diabetic controls (68.7 µV, $p=0.01$) and subjects with early maculopathy (74.5 µV, $p<0.01$). There was a trend towards prolonged OP IT in subjects with sight-threatening maculopathy (18.8 ms) compared to healthy controls (17.7 ms, $p<0.02$). IT was non-significantly prolonged compared to diabetic controls (18.6 ms, $p>0.50$) and subjects with early maculopathy (18.0 ms, $p>0.20$). However there was no significant difference between healthy controls, diabetic controls and subjects with early maculopathy. This suggests that OP function is well preserved until later stages of disease. Similar results have been previously reported.[152,208,209]

In some studies prolongation of OP IT has only been reported in diabetic subjects with no DR.[201,208] In another study only sum OP amplitude was reduced; summed OP IT was prolonged but not the 1st peak IT.[210] However no adjustments for age or systemic risk factors were made in these studies.

6.5.5 Contrast sensitivity

In my study contrast sensitivity (CS) was reduced in early maculopathy compared to healthy controls, and in sight-threatening maculopathy compared to the other three groups. There was no significant difference between healthy and diabetic controls. CS disruption has been reported prior to development of vasculopathy,[209] in early DR,[211,212] and in ischaemic maculopathy.[81] However Jackson et al (2012) reported no difference between healthy and diabetic controls.[213]

One study reported a reduction in CS in subjects with no DR.[80] However this study only included subjects aged 55-75 years, and mean duration of DM was 16 years. Also, this study utilised a computerised method of testing and only noted changes in mesopic conditions.

6.5.6 Systemic risk factors

In my study subjects with early and sight-threatening maculopathy had a significantly greater HbA1c and longer duration of DM as compared to diabetic controls, and subjects with DM were more likely to be on anti-hypertensive and

lipid lowering agents. As described in Chapter 3, the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) reported that duration of DM and elevated HbA1c were risk factors for the development of diabetic macular oedema.[69] The Early Treatment of Diabetic Retinopathy Study (ETDRS) reported that elevated cholesterol levels was associated with an increased likelihood of developing hard exudates and visual loss[119] and the use of lipid lowering agents in the Effect of Fenofibrate on the need for Laser treatment for Diabetic retinopathy (FIELD) study was associated with a reduced risk of developing macular oedema.[123] In the UK Prospective Diabetes Study (UKPDS) group tighter BP control was associated with a reduced risk of developing retinal hard exudates and a reduced need for laser for treatment of diabetic maculopathy.[214] Therefore the differences in systemic risk factors between the groups are not unexpected and my cohort appears to be representative of patients seen in clinical practice.

6.5.7 Ischaemic maculopathy

As seen in the sub-analysis of the sight-threatening maculopathy group (Section 6.3.7 and Table 6.14), subjects with ischaemic macular changes had reduced retinal function, compared to subjects with only CSMO, across all functional assessments. Statistically significant worsening was reached in BCVA (16% reduction), CS (16% reduction), MP central ring sensitivity (52% reduction) and mfERG central ring amplitude (64% reduction). These findings are important as many recent studies do not include fluorescein angiography (FA) for determination of the foveal avascular zone as part of the assessment of macular function.[147-149,155,157,167,168]

In current clinical practice, diagnosis of CSMO is based upon clinical findings with OCT highlighting the extent or severity of macular oedema. Therefore I believe the sight-threatening maculopathy group appears to be representative of those patients whom we encounter in clinical practice. I excluded subjects who required treatment for their maculopathy at baseline. So my cohort does not include subjects with the worst disease. It is likely that inclusion of those with worst disease may have produced reduced mean amplitude and prolonged IT in the sight-threatening maculopathy group.

6.5.8 Diagnosing disease using tests of macular function

In my analysis of association, I demonstrated that reduced function in BCVA, CS, MP, mfERG central ring amplitude and OP IT were significantly associated with a diagnosis of sight-threatening maculopathy ($p<0.03$ for all comparisons). There was a trend towards reduced function in mfERG IT ($p>0.05$) but there was no significance with OP sum amplitude ($p>0.10$).

There has been only one study that has assessed the predictive power of tests of central macular function in subjects with diabetes mellitus. Al Shafaee et al (2011) assessed MP in an Omani population, comparing healthy controls, subjects with DM and subjects with abnormal blood sugar levels that does not meet criteria for DM (pre-diabetes).[184] The authors reported that MP was sensitive in differentiating between subjects with DM and healthy controls ($p=0.001$); and between subjects with pre-diabetes and healthy controls ($p<0.01$). Further sub-analysis demonstrated that MP was sensitive in determining between subjects with DM and DR, and subjects with DM but no DR ($p<0.001$). There was no difference between subjects with DM and subjects with pre-diabetes ($p>0.50$). The authors did not conclude on these findings. However the authors did not report their critical value nor have they reported sensitivity and specificity.

Using a single investigation to determine reduced function in sight-threatening maculopathy, sensitivity was weak (33.3-55.3%) but specificity was moderate (73.3-89.4%). By combining two investigations, sensitivity falls further (17-5-28.9%) and specificity improves (87.0-100%). There have been no studies that have analysed sensitivity and specificity of MP, mfERG or OP as predictive biomarkers for sight-threatening maculopathy. My results suggest that tests of central macular function would be more beneficial in determining early or no disease rather than sight-threatening maculopathy. By combining investigations specificity improves, suggesting the use of multiple investigations would be more beneficial.

Using a single investigation, the positive predictive value (PPV, 61.9-78.3%) and negative predictive value (NPV, 58.1-66.0%) demonstrated moderate probability in determining if a subject has sight-threatening maculopathy. By combining two

investigations, there was only a small change in PPV (64.7-100%) and NPV (54.2-61.1%). There have been no studies that have analysed the predictive value of these tests. My results suggest that these tests offer a moderate predictive value in predicting presence of sight-threatening maculopathy.

These results in specificity, sensitivity, PPV and NPV should be treated with some caution. The study was not powered for such analysis. The critical value chosen may not be applicable to other studies or cohorts due to use of local protocols in data collection. However, with the interest in predictive and prognostic biomarkers for macular disease and DR, my study demonstrate that CS, MP, mfERG and OP offer an opportunity to quantify disease severity. I discuss the potential benefits of these investigations in Chapter 8

6.5.9 Limitations of the study

In my study I excluded any potential subjects with CSMO who required treatment for their maculopathy within three months of entering the study. Thus it is likely that the reduction in retinal function in the sight-threatening maculopathy group would have been greater had I included those subjects in the study. DM is a multisystem disease with varying levels of morbidity depending on severity of disease. Subjects with worse disease may have been inadvertently excluded from the study as they may have been unable to undertake the investigations or were unwilling due to poor health.

Sample size calculations recommended individual group sizes of 30 subjects. This was not achieved in the diabetic controls, early maculopathy and ischaemic maculopathy group despite the recruitment period being extended. Therefore my analyses may be underpowered. The difficulty in recruitment of subjects with ischaemic maculopathy was predominantly due to previous macular treatment. Nevertheless, the reduction in retinal function across all assessments suggests that a larger cohort may not have added any more strength to the results gathered.

Recruitment took place over two periods due to changes in personnel and need for more healthy and diabetic controls. Nevertheless, I was involved in all data

collection and analysis to ensure continuity in interpretation of the raw data and all were subjected to the same conditions during the study.

Subjects with DM were already on systemic treatment prior to entering the study, hence similarities in serum cholesterol and blood pressure. However I do not have any information on prior systemic control with which to compare retinal function.

6.6 Summary

In this chapter I have demonstrated that sight-threatening maculopathy is associated with reduced central macular function over a variety of measures of function (BCVA, CS, MP central ring sensitivity, mfERG central ring amplitude, mfERG central ring IT and OP sum amplitude). In addition there appears to be reduced macular function in early maculopathy in investigations of CS, MP and mfERG central ring amplitude. I was unable to demonstrate any significant difference in central macular function between healthy controls and diabetic controls. This suggests that these investigations may help determine severity of disease in conjunction with current clinical techniques of assessing diabetic maculopathy. In addition, the reasonably high specificity levels of these investigations may aid in excluding subjects with severe diabetic maculopathy. In the next chapter I will present the results of the longitudinal investigations to determine change in central macular function in subjects with diabetic maculopathy and in Chapter 8 I will explore the potential role of visual function assessment in the management of diabetic maculopathy.

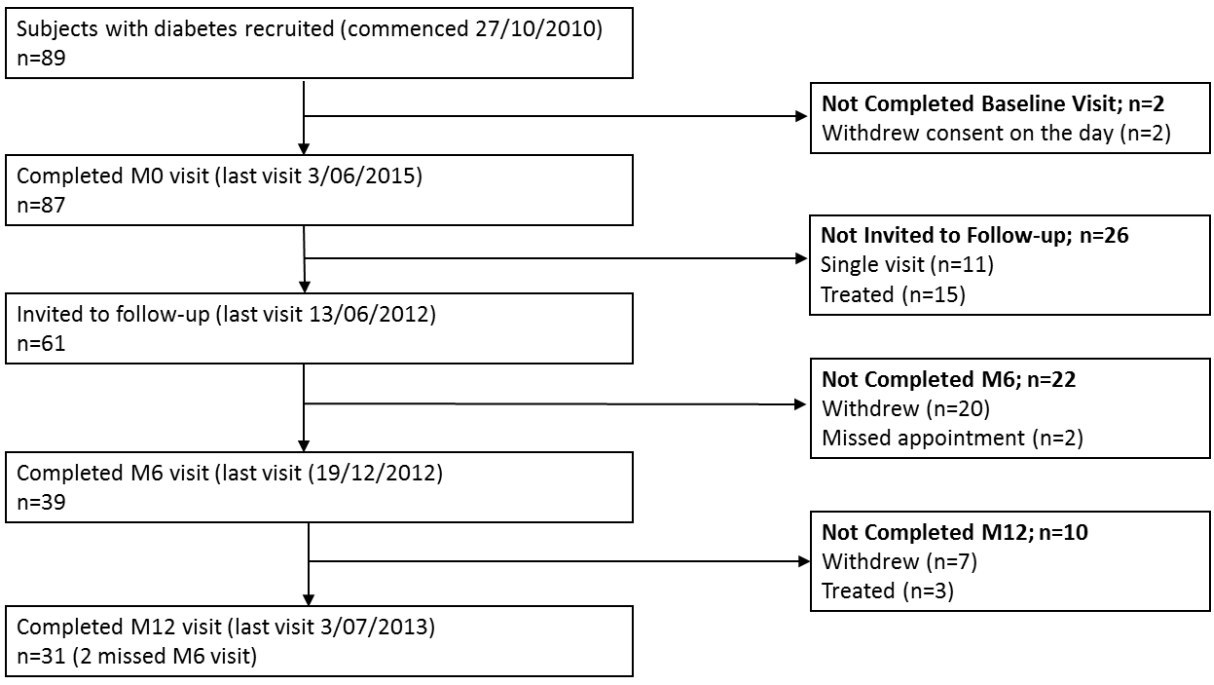
Chapter 7 Results – longitudinal

In this chapter I present the results of the longitudinal data. I describe the follow up of those subjects described in Chapter 6, and explore the reasons for those who exited the study. I present the longitudinal results of the structural and functional assessments described in Chapter 4.

7.1 Follow up

As described in Chapter 6, a total of 118 subjects were recruited to my study. Of these there were 89 subjects with diabetes mellitus (DM) who were all considered for the longitudinal study. In Figure 7.1 I present the consort style diagram that summarises the allocation of the subjects with DM. Of these 89 subjects, 2 subjects did not complete the baseline visit, 11 were recruited during the second phase of recruitment, and 15 subjects received treatment for their maculopathy. Therefore, 61 subjects (70%) were invited to follow up. Of these 39 (64%) completed the month 6 visit and 31 (51%) completed the month 12 visit.

Figure 7.1: Consort style diagram summarising the allocation of subjects with DM at 6 months and 12 months



Subjects with DM were divided into the three groups as follows: diabetic controls, n=4 and n=4 at 6 and 12 months respectively; early maculopathy, n=17 and n=13; sight-threatening maculopathy, n=18 and n=14 (Table 7.1).

Table 7.1: Distribution across groups for 89 baseline subjects with DM for follow-up. Outcome at month 6 and month 12 represents reasons subjects who did not attend for that particular visit. The missed visit represents the two subjects who did not attend the month 6 visit but did attend for month 12.

Time point	Outcome	Number of subjects		
		Diabetic Controls	Early Maculopathy	Sight-threatening maculopathy
Baseline	Recruited	24	24	41
Month 6	Completed	4	17	18
	Single visit	10	0	1
	Treated	0	0	15
	Withdrew	6	4	6
	No reason given	3	2	1
	Missed visit	1	1	0
Month 12	Completed	4	13	14
	Treated	0	1	2
	Withdrew	1	4	2

Between the baseline and month 6 visit, 20 subjects withdrew from the study and a further two subjects missed their appointment. Therefore 39 of the invited subjects (64%) completed a month 6 visit. Subjects either declined to take further part in the study or failed to attend their appointment. If subjects failed to attend then a further appointment was made and the subject contacted. If the subject again failed to attend, then they were withdrawn from the study.

Of those who completed the month 6 visit, 10 subjects did not attend for the month 12 visit. Therefore, 31 of the invited subjects (31/61, 51%) completed the month 12 visit. Three of these subjects received treatment for their maculopathy while seven

subjects declined to take further part in the study and so were withdrawn. Two subjects who did not attend for the 6 month visit agreed to complete the month 12 visit.

7.2 Demographic analysis

I performed a comparison of the demographic data between subjects who completed the month 6 and month 12 visits with those who exited the study prior to these time points.

7.2.1 Comparison at month 6

In Table 7.2 I present a comparison of the demographic variables (age, serum HbA1c, serum cholesterol, systolic and diastolic blood pressure), best corrected visual acuity (BCVA) and contrast sensitivity (CS) between subjects who completed the month 6 visit and those who exited the study prior to the month 6 visit. For the subjects who exited the study I have separated the analysis between those who required treatment for their maculopathy (Treated) and those who no longer wanted to continue with the study (Withdrew).

Between all three groups there were no significant differences in age, serum cholesterol, and systolic and diastolic BP at baseline ($p>0.10$ for all comparisons). There were significant differences between the groups for serum HbA1c ($p<0.05$), BCVA ($p<0.01$) and CS ($p<0.01$). Analysis was performed using ANOVA, with $p<0.05$ deemed to be significant. Post-hoc comparison, using Sidak, was then performed to compare between the three groups.

Subjects who received treatment prior to the month 6 visit had a significantly greater mean HbA1c at baseline than subjects who withdrew from the study but did not receive treatment ($p<0.05$); mean HbA1c was also increased compared to those who completed the study, though this was not significant ($p>0.20$) (Table 7.3). Subjects who were treated also had a significantly reduced BCVA (mean reduction of 9%, $p<0.05$) and CS (mean reduction of 13%, $p<0.01$) as compared to the other two groups. There was no significant difference between subjects who completed the study and those who withdrew but were not treated.

Table 7.2: Intergroup comparison of demographic variables at baseline between subjects who completed the study, those who were treated and those who were withdrawn without treatment prior to the month 6 visit. BP = blood pressure; BCVA = best corrected visual acuity; CS = contrast sensitivity; SD = standard deviation. *P<0.05 was deemed significant.

Variable	Completed (n=39)	Treated (n=15)	Withdrew (n=35)	P Value
Mean age at baseline (years) (SD)	57.5 (10.6)	51.7 (11.7)	56.8 (14.1)	0.29
Mean serum HbA1c (%) (SD)	8.3 (1.6)	9.1 (2.6)	7.6 (1.5)	0.03*
Mean serum cholesterol (mmol/L) (SD)	4.5 (1.1)	4.6 (1.1)	4.6 (1.1)	0.82
Mean systolic BP (mmHg) (SD)	135.7 (15.1)	131.5 (15.1)	132.3 (14.7)	0.52
Mean diastolic BP (mmHg) (SD)	76.2 (8.3)	74.3 (9.8)	79.3 (8.8)	0.13
Mean BCVA (letters) (SD)	84.7 (6.7)	77.2 (8.6)	84.4 (9.4)	<0.01*
Mean CS (letters) (SD)	37.6 (3.7)	32.5 (4.4)	37.5 (4.3)	<0.01*

Table 7.3: Post-hoc sub-analysis of baseline demographic variables, using SIdak, of subjects who were treated prior to the month 6 visit compared to those who either completed the study or withdrew but did not receive treatment. CL = confidence limits. *P values <0.05 are deemed significant.

	Treated	Completed		Withdrew	
	Mean value (SD) Range	Mean difference (95% CL)	P value	Mean difference (95% CL)	P value
Serum HbA1c (%)	9.1 (1.6) 6.4-13.7	-0.7 (-0.5, 2.0)	>0.20	-1.4 (-2.7, -0.1)	<0.05*
BCVA (letters)	77.2 (8.5) 57-89	7.5 (1.5, 13.6)	<0.01*	7.2 (1.1, 13.4)	<0.05*
CS (letters)	32.5 (4.4) 24-39	5.2 (2.2, 8.2)	<0.01*	5.0 (1.9, 8.1)	<0.01*

Subjects who completed the study were not significantly different from those who withdrew with respect to their demographic variables. The significant difference was the poorer control of DM and reduced visual function in subjects who required treatment. This would suggest that any bias introduced by subjects being withdrawn could be small.

7.2.2 Comparison at month 12

In Table 7.4 I present a comparison of demographic variables between subjects who completed the study at month 12 and those who did not. Once again I have separated the group who did not complete the study into those requiring treatment and those who no longer wanted to continue.

Similar to the comparison at month 6, there were no significant differences in age, serum cholesterol and systolic and diastolic BP between the three groups, but there were differences between serum HbA1c, BCVA and CS.

Table 7.4: Intergroup comparison of demographic variables at baseline between subjects who completed the study and those who were treated or withdrawn without treatment prior to the month 12 visit. BP = blood pressure; BCVA = best corrected visual acuity; CS = contrast sensitivity; SD = standard deviation. *P<0.05 was deemed significant.

Variable	Completed (n=31)	Treated (n=18)	Withdrew (n=40)	P Value
Mean age (years) (SD)	59.3 (11.8)	52.0 (10.6)	55.8 (13.0)	0.13
Mean serum HbA1c (%) (SD)	8.4 (1.6)	9.0 (2.2)	7.7 (1.5)	0.02*
Mean serum cholesterol (mmol/L) (SD)	4.6 (1.2)	4.7 (1.0)	4.5 (1.0)	0.77
Mean systolic BP (mmHg) (SD)	136.0 (15.9)	130.7 (14.8)	133.2 (14.3)	0.47
Mean diastolic BP (mmHg) (SD)	74.7 (8.7)	75.7 (9.9)	79.6 (8.0)	0.06
Mean BCVA (letters) (SD)	85.5 (6.6)	79.1 (9.0)	83.6 (9.2)	0.04*
Mean CS (letters) (SD)	38.0 (3.9)	33.6 (4.9)	37.1 (4.1)	<0.01*

Post-hoc analysis, using Sidak, demonstrated no significant difference between subjects who completed the study and those who withdrew but did not receive

treatment (Table 7.5). As at month 6, subjects who were treated had a higher HbA1c than the other two groups, and this was significant compared to those who withdrew (14% higher in those treated, $p<0.05$).

BCVA was significantly reduced in subjects treated compared to those who completed the study (reduction of 8%, $p<0.05$). CS was significantly reduced compared to those who completed the study (reduction of 13%, $p<0.01$) and those who withdrew but were not treated (reduction of 10%, $p<0.02$).

Table 7.5: Post-hoc sub-analysis of baseline demographic variables, using SIdak, of subjects who were treated prior to the 12 month visit to those who either completed the study or withdrew but did not receive treatment. CL = confidence limits. *P values <0.05 are deemed significant and compare each group to those treated.

	Treated	Completed		Withdrew	
	Mean value (SD) Range	Mean difference (95% CL)	P value	Mean difference (95% CL)	P value
Serum HbA1c (%)	9.0 (2.2) 5.6 – 13.7	-0.5 (-1.7, 0.7)	0.68	-1.3 (-2.4, -0.1)	0.03*
BCVA (letters)	79.1 (9.0) 57 - 93	6.4 (0.4, 12.4)	<0.04*	4.6 (-1.2, 10.4)	0.16
CS (letters)	33.6 (4.9) 24 - 41	4.4 (1.4, 7.4)	<0.01*	3.4 (0.5, 6.4)	<0.02*

In summary, there was no significant difference in systemic risk factors between subjects who completed the study and those who withdrew before the month 6 and month 12 visits. This would suggest that any bias introduced by subjects falling out of the study by month 12 could be small. The only significant difference was between subjects who exited the study for treatment of their maculopathy and the subjects who withdrew or completed the study. The worse serum HbA1c, BCVA and CS are a reflection of poor control of DM that has resulted in development of maculopathy and our current clinical pathway in which decrease in visual function triggers the clinical decision to commence treatment.

7.3 Longitudinal assessment of central macular function and structure

In the following tables I present the longitudinal results of my study. Data are presented as means throughout. I have used the Wilcoxon nonparametric test due to the low number of subjects in each group. I have also used the paired Student t-test to compare between subjects with early maculopathy and subjects with sight-threatening maculopathy. This was done as there were similar numbers in each group and to consolidate the findings with the Wilcoxon test.

In the tables I firstly present the results for the group as a whole, i.e. all subjects who completed the month 6 and month 12 visits. I have reported the mean baseline value and then the mean change from baseline. $P < 0.01$ was deemed significant for analyses of the five primary outcomes of microperimetry (MP) central ring sensitivity, multifocal electroretinogram (mfERG) central ring amplitude, mfERG implicit time (IT), oscillatory potential (OP) sum amplitude and OP IT following Bonferroni correction. $P<0.05$ was deemed significant for analyses of BCVA and CS. I have also reported the number of participants whose recordings have increased, decreased or stayed the same compared to the baseline value. This allows the reader to identify trends in change as well as identify outliers which may affect the overall mean. I then performed a sub-analysis of each group, reporting the results in the same manner as described above.

7.3.1 Best corrected visual acuity

At month 6 there was no clinically meaningful change in mean BCVA (-0.5 letters) and this was statistically non-significant (p>0.20) (Table 7.5). Just as many subjects gained VA as compared to those who lost VA. Subgroup analysis revealed no clinically significant change in BCVA (-<1 letter) for both the early maculopathy and sight-threatening maculopathy groups, and this was not significant (p>0.20 for both comparisons).

At month 12 the findings were very similar. There was no clinically meaningful change in BCVA (mean -0.4 letters) and this was not statistically significant (p≥0.50) (Table 7.5). Subgroup analysis revealed no clinically significant change in BCVA (mean -<1 letter) for both maculopathy groups though again this was not statistically significant (p>0.20 for both comparisons)

7.3.2 Contrast sensitivity

The results for Pelli-Robson contrast sensitivity performed with standardised visions by accredited optometrist observers are shown in Table 7.6.

At month 6 there was a clinically insignificant change in CS (mean 0.2 letters) but this was not significant (p>0.50). Sub-group analysis revealed a clinically insignificant change in both maculopathy groups (mean <1 letter) but this was not significant (p>0.20 for both comparisons).

At month 12 there was a clinically insignificant worsening in CS (mean -0.5 letters) but this was not significant (p>0.50). Sub-group analysis showed no particular pattern with slight improvement in the early maculopathy group (0.2 letters) but worsening in the sight-threatening maculopathy group (-0.7 letters). Both outcomes were clinically insignificant and the comparisons were not significant (p>0.20).

Table 7.5: Mean change from baseline in best corrected visual acuity (letters) at month 6 and month 12. Group and sub-group analysis were performed using Wilcoxon test. Change from baseline reported as number of subjects who gained (+) or lost (-) function, or stayed the same (none). *P<0.05 was deemed significant. SD = standard deviation. One subject did not attend for BCVA assessment at month 6

	All		Diabetic controls (n=4 at month 6) (n=4 at month 12)		Early maculopathy (n=16 at month 6) (n=13 at month 12)		Sight-threatening maculopathy (n=18 at month 6) (n= 14 at month 12)	
Best corrected visual acuity (letters)	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value
month 6 (n=38)	85.0 (6.8) 68-95	-0.5 (17,17,4) 0.48	82.0 (9.4) 68-93	1.2 (2,1,1) 0.72	85.6 (4.7) 79-95	-0.8 (6,8,2) 0.45	85.3 (7.7) 68-95	-0.6 (9,8,1) 0.62
month 12 (n=31)	85.5 (6.6) 68-95	-0.4 (10,16,5) 0.50	83.0 (3.3) 79-87	1.5 (2,2,0) 0.47	86.5 79-95	-0.5 (4,6,3) 0.68	85.2 (8.5) 68-95	-0.9 (4,8,2) 0.27

Table 7.6: Mean change from baseline in contrast sensitivity (letters) at month 6 and month 12. Group and sub-group analysis were performed using Wilcoxon test. Change from baseline reported as number of subjects who gained (+) or lost (-) function, or stayed the same (none).* P<0.05 was deemed significant. SD = standard deviation

	All		Diabetic controls (n=4 at month 6) (n=4 at month 12)		Early maculopathy (n=17 at month 6) (n=13 at month 12)		Sight-threatening maculopathy (n=18 at month 6) (n= 14 at month 12)	
Contrast sensitivity (letters)	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value
month 6 (n=39)	37.5 (3.8) 26-42	0.2 (16,12,11) 0.70	39.0 (3.3) 35-42	-0.6 (1,3,0) 0.50	37.5 (3.8) 30-42	0.7 (7,3,7) 0.37	37.1 (4.0) 26-42	0.1 (8,6,4) 0.80
month 12 (n=31)	38.0 (3.9) 26-42	-0.5 (13,12,6) 0.59	39.5 (2.9) 36-42	-1.5 (1,2,1) 0.59	38.2 (4.0) 30-42	0.2 (8,3,2) 0.56	37.4 (4.3) 26-42	-0.7 (4,7,3) 0.45

7.3.3 Microperimetry

At month 6 there was a trend towards improved function across the whole group (p=0.02) (Table 7.7). Nearly two-thirds of subjects showed improved function (23/36, 64%). Sub-analysis revealed that each group showed improvement in function, though none of the groups demonstrated statistical significance, p>0.01 for all comparisons. Subjects with sight-threatening maculopathy showed a greater mean improvement than subjects with early maculopathy (0.8 dB vs 0.3 dB).

At month 12, mean sensitivity had significantly improved across the whole group (mean change of 1.7 dB, p<0.01) (Table 7.7). Over 80% (23/28) had shown an improvement in function. Sub-analysis revealed that each group showed an improvement. Subjects with sight-threatening maculopathy showed a significant mean improvement of 13% (1.9 dB, p<0.01). Subjects with early maculopathy showed a trend towards improved function of 10% (mean change of 1.6 dB, p=0.02). Paired t-test demonstrated similar p values. All but two of the sight-threatening maculopathy group (11/13, 85%) and 75% (9/12) of the early maculopathy group showed an improvement.

7.3.4 Multifocal electroretinogram central ring amplitude

At month 6 there was a small but non-significant reduction in mean central ring amplitude in the whole group (mean -0.9 nV/deg^2, p>0.20) (Table 7.8). This is reflected in that just over half (54%) showed improvement in amplitude whilst 46% showed worsening amplitude. Sub-group analysis revealed no specific pattern with a decrease in function in the early maculopathy group (mean change of -5.4 nV/deg^2, p>0.20) and a minimal improvement in the sight-threatening maculopathy group (mean change of 0.5 nV/deg^2, p>0.50). Paired t-test was also non-significant (p>0.20 for all comparisons).

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Table 7.7: Mean change from baseline in microperimetry central ring sensitivity at month 6 and month 12. Group and sub-group analysis were performed using Wilcoxon test. Change from baseline reported as number of subjects who gained (+) or lost (-) function, or stayed the same (none). *P<0.01 was deemed significant. SD = standard deviation. Three subjects were excluded as MP was not working

	All		Diabetic controls (n=4 at month 6) (n=3 at month 12)		Early maculopathy (n=15 at month 6) (n=12 at month 12)		Sight-threatening maculopathy (n=17 at month 6) (n= 13 at month 12)	
MP central ring sensitivity (dB)	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value
month 6 (n=36)	15.4 (2.8) 7.4-20.0	0.6 (23,11,2) 0.02	17.4 (1.3) 16.0-18.8	0.90 (2,2,0) 0.58	15.5 (2.9) 9.8-20.0	0.3 (10,3,2) 0.2	14.9 (2.9) 7.4-19.2	0.8 (11,6,0) 0.08
month 12 (n=28)	15.7 (2.9) 7.4-20.0	1.7 (23,5,0) <0.01*	17.8 (1.1) 16.6-18.8	1.3 (3,0,0) 0.11	15.8 (2.8) 9.8-20.0	1.6 (9,3,0) 0.02	15.0 (3.2) 7.4-19.2	1.9 (11,2,0) <0.01*

Table 7.8: Mean change in mfERG central ring amplitude at month 6 and month 12. Group and sub-group analyses were performed using Wilcoxon test. Change from baseline reported as number of subjects who gained (+) or lost (-) function, or stayed the same (none). *P<0.01 was deemed significant to correct for multiple comparisons. SD = standard deviation

	All		Diabetic controls (n=4 at month 6) (n=4 at month 12)		Early maculopathy (n=17 at month 6) (n=13 at month 12)		Sight-threatening maculopathy (n=18 at month 6) (n= 14 at month 12)	
mfERG central ring amplitude (nV/deg^2)	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value
month 6 (n=39)	53.3 (20.0) 4.5-91.2	-0.9 (21,18,0) 0.96	57.7 (18.6) 34.1-83.6	8.6 (3,1,0) 0.50	59.9 (16.7) 33.1-91.0	-5.4 (7,10,0) 0.28	46.7 (21.5) 4.5-91.2	0.5 (11,7,0) 0.69
month 12 (n=31)	50.2 (18.7) 4.5-88.5	-5.0 (11,20,0) 0.15	56.7 (22.9) 34.1-88.5	-8.3 (2,2,0) 0.72	54.2 (14.8) 32.1-75.3	-7.9 (4,9,0) 0.15	44.7 (20.6) 4.5-80.9	-3.3 (5,9,0) 0.36

At month 12, amplitude worsened on average by 5.0 nV/deg^2 though this was not statistically significant (p>0.10) (Table 7.8). Just under two-thirds of subjects showed a reduction in amplitude (20/31). Sub-group analysis revealed that all groups showed a mean reduction in amplitude though none of them reached statistical significance (p>0.10 for all comparisons). Decrease in amplitude was similar between the diabetic control (mean change of -8.3 nV/deg^2, -15%) and the early maculopathy (mean change of -7.9 nV/deg^2, -15%) groups. Subjects with sight-threatening maculopathy showed a smaller reduction in amplitude (mean change of-3.3 nV/deg^2, -7%). Paired t-test confirmed this reduction was statistically not significant (p>0.20 for all comparisons).

7.3.5 Multifocal electroretinogram central ring implicit time

At month 6, there was no change in mean mfERG implicit time (IT) in the group as a whole (mean 0.0 ms, p>0.50) (Table 7.9). Similar numbers of subjects showed either prolongation (36%) or improvement (43%) of implicit time. Sub-group analysis demonstrated prolongation in both the diabetic control group (mean change of 1.4 ms, p>0.20) and the early maculopathy group (mean change of 0.9 ms, p>0.50), though none reached statistical significance. There was a mean improvement in implicit time in the sight-threatening maculopathy group, though this again did not reach statistical significance (mean change of -1.1 ms, p≥0.20).

At month 12, there was an overall small increase in mfERG IT though this did not reach statistical significance (mean change of 0.4 ms, p>0.50) (Table 7.9). Just as many subjects showed prolongation of IT (42%) as those who showed an improvement (39%). Sub-group analysis showed a similar pattern to that at month 6. Diabetic controls showed a small prolongation in IT (mean change of 0.4 ms, p>0.20), as did the early maculopathy group (mean change of 0.9 ms, p>0.20). There was an improvement in IT in the sight-threatening maculopathy group (mean change of -0.9 ms, p>0.50), though this did not reach statistical significance.

Table 7.9: Mean change in mfERG central ring implicit time at 6 months and 12 months. Group and sub-group analyses were performed using Wilcoxon test. Change from baseline reported as number of subjects who showed prolongation (+) or improvement (-) in IT, or stayed the same (none). *P<0.01 was deemed significant. SD = standard deviation

	All		Diabetic controls (n=4 at month 6) (n=4 at month 12)		Early maculopathy (n=17 at month 6) (n=13 at month 12)		Sight-threatening maculopathy (n=18 at month 6) (n= 14 at month 12)	
mfERG central ring implicit time (ms)	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value
month 6 (n=39)	39.6 (3.4) 31.4–50.0	0.0 (14,17,8) 0.83	38.1 (1.3) 37.3-40.2	1.4 (2,1,1) 0.36	39.1 (2.5) 36.3-44.1	0.9 (7,5,5) 0.56	40.5 (4.3) 31.4-50.0	-1.1 (5,11,2) 0.20
month 12 (n=31)	39.7 (3.5) 31.5-50.0	0.4 (13,12,6) 0.60	39.3 (2.4) 37.3-42.2	0.5 (2,1,1) 0.41	38.9 (2.2) 36.3-44.1	0.9 (7,4,2) 0.42	40.7 (4.6) 31.4-50.0	-0.9 (4,7,3) 0.65

7.3.6 Oscillatory potential sum amplitude

At month 6, there was an overall worsening of OP sum amplitude of 12%, though this did not reach statistical significance (mean change of -6.9 μV, p>0.10) (Table 7.10). Just over half of subjects showed worsening amplitude (59%) with the rest showing an improvement. Sub-group analysis demonstrated worsening amplitude in all groups though none reached statistical significance. Early maculopathy group showed a mean worsening in amplitude of 22% (-14.9 μV, p>0.05). There was only a minimal worsening in amplitude of 2% in the sight-threatening maculopathy group (mean change of -0.8 μV, p>0.50).

At month 12, there was a trend towards worsening amplitude of 11% for the whole group (mean change of -6.2 μV, p<0.05) (Table 7.10). Over two-thirds showed worsening amplitude (68%). Sub-group analysis revealed a similar worsening in amplitude of 9% and 10%, respectively, in both the early maculopathy (mean change of -5.6 μV, p>0.20) and the sight-threatening maculopathy group (mean change of -4.5 μV, p>0.05). The greatest decrease in function was seen in the diabetic control group with a mean reduction in function of 20% though this did not reach significance (mean change of -14.2 μV, p>0.20).Similar p values were seen in the paired t-test.

Table 7.10: Mean change in OP sum amplitude from baseline at month 6 and month 12. Group and sub-group analysis were performed using Wilcoxon test. Change from baseline reported as number of subjects who gained (+) or lost (-) function, or stayed the same (none). *P<0.01 was deemed significant. SD = standard deviation

	All		Diabetic controls (n=4 at month 6) (n=4 at month 12)		Early maculopathy (n=17 at month 6) (n=13 at month 12)		Sight-threatening maculopathy (n=18 at month 6) (n= 14 at month 12)	
OP sum amplitude (μV)	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value
month 6 (n=39)	57.6 (26.0) 16.9-127.2	-6.9 (16,23,0) 0.14	71.2 (37.1) 23.2-111.0	-9.4 (2,2,0) 0.69	69.3 (31.0) 19.0-154.0	-14.9 (6,11,0) 0.09	45.7 (16.7) 16.9-82.1	-0.8 (8,10,0) 0.87
month 12 (n=31)	55.3 (23.1) 16.9-101.0	-6.2 (10,21,0) 0.03	71.9 (34.0) 23.2-101.0	-14.2 (2,2,0) 0.47	60.7 (20.8) 19.0-98.5	-5.6 (4,9,0) 0.31	45.6 (18.8) 16.9-82.1	-4.5 (4,10,0) 0.08

7.3.7 Oscillatory potential 1st peak implicit time

At month 6 there was a minimal prolongation in OP IT which was statistically not significant (mean change of 0.1 ms, p>0.20) (Table 7.11). Over half (20/38) showed no change in their OP IT. Sub-group analysis revealed that all groups showed prolongation in OP IT though none reached statistical significance (p>0.20 for all comparisons). Both the early maculopathy group and the sight-threatening maculopathy group showed only a minimal prolongation of IT (mean change of 0.1 ms). Diabetic controls showed the greatest prolongation (mean change of 0.6 ms).

At month 12 there was an overall mean improvement in OP IT though this was not significant (-0.3 ms, p>0.50) (Table 7.11). Just as many subjects showed prolongation in IT (27%) as those who showed an improvement in it (23%), with half of all subjects demonstrating no change (15/30). Sub-group analysis revealed that the improvement in OP IT was driven by the early maculopathy group which showed a mean improvement of 1.0 ms, though this was not statistically significant (p>0.20). The sight-threatening maculopathy group again showed a small prolongation in IT that was not statistically significant (mean change of 0.2 ms, p>0.50). Once again the greatest prolongation was in the diabetic control group, though this was not statistically significant (mean change of 0.8 ms, p>0.20).

Table 7.11: Mean change in OP 1st peak implicit time at 6 months and 12 months. Group and sub-group analysis were performed using Wilcoxon test. Change from baseline reported as number of subjects who showed prolongation (+) or improvement (-) in IT, or stayed the same (none). P<0.01 was deemed significant. SD = standard deviation

	All		Diabetic controls (n=4 at month 6) (n=4 at month 12)		Early maculopathy (n=16 at month 6) (n=13 at month 12)		Sight-threatening maculopathy (n=18 at month 6) (n= 13 at month 12)	
OP 1st peak implicit time (ms)	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value
month 6 (n=38)	18.1 (0.9) 17.0–23.0	0.1 (10,8,20) 0.39	17.8 (0.8) 17.0-23.0	0.6 (2,1,1) 0.28	18.1 (1.0) 17.0-20.0	0.1 (2,1,13) 0.79	18.3 (0.9) 17.0-20.0	0.1 (6,6,6) 0.80
month 12 (n=30)	18.2 (0.9) 17.0-20.0	-0.3 (8,7,15) 0.90	18.0 (0.9) 17.0-19.0	0.8 (2,1,1) 0.41	18.2 (0.9) 17.0-20.0	-1.0 (3,3,7) 0.34	18.3 (1.0) 17.0-20.0	0.2 (3,3,7) 0.74

7.3.8 Optical coherence tomography

At month 6 there was an overall decrease in mean central subfield thickness (CSFT) though this was not significant (-11.1 µm, p>0.50) (Table 7.12). This decrease was predominantly driven by the sight-threatening maculopathy group, with a mean reduction in CSFT of 36 µm though this was not statistically significant (p>0.20). A non-statistically significant increase in CSFT was seen in both the diabetic control group (mean change of 6%, p>0.20) and the early maculopathy group (mean change of 4%, p>0.20).

At month 12 there was an overall mean increase in CSFT of 8.2 µm though this was not statistically significant (p>0.50) (Table 6.12). Just over half of subjects (18/31) showed an increase in CSFT with the rest showing a decrease. Sub-group analysis demonstrated that increase in CSFT was seen in all groups though none reached statistical significance (p>0.20 for all comparisons). Diabetic controls had a mean increase of 2% in CSFT, early maculopathy group of 5%, and sight-threatening maculopathy group of 2%.

Table 7.12: Mean change in CSFT at 6 months and 12 months. Group and sub-group analysis were performed using Wilcoxon test. Change from baseline reported as number of subjects who showed an increase (+) or a decrease (-) in CSFT, or stayed the same (none). *P<0.05 was deemed significant. SD = standard deviation

	All		Diabetic Controls (n=4 at month 6) (n=4 at month 12)		Early Maculopathy (n=16 at month 6) (n=13 at month 12)		Sight-threatening maculopathy (n=18 at month 6) (n= 14 at month 12)	
Central subfield thickness (µm)	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value	Baseline mean (SD) range	Mean change (+,-,none) p value
month 6 (n=38)	288.3 (42.3) 190–399	-11.1 (18,16,4) 0.97	249.8 (23.1) 211-270	13.8 (2,1,1) 0.27	276.1 (35.3) 190-349	10.6 (10,4,2) 0.30	308.7 (41.7) 246-399	-36.0 (6,11,1) 0.21
month 12 (n=31)	280.7 (34.3) 190-370	8.2 (18,13,0) 0.90	263.0 (13.0) 248-277	5.5 (2,2,0) 0.47	269.2 (31.4) 190-324	12.9 (8,5,0) 0.31	295.9 (35.9) 246-370	4.6 (8,6,0) 1.00

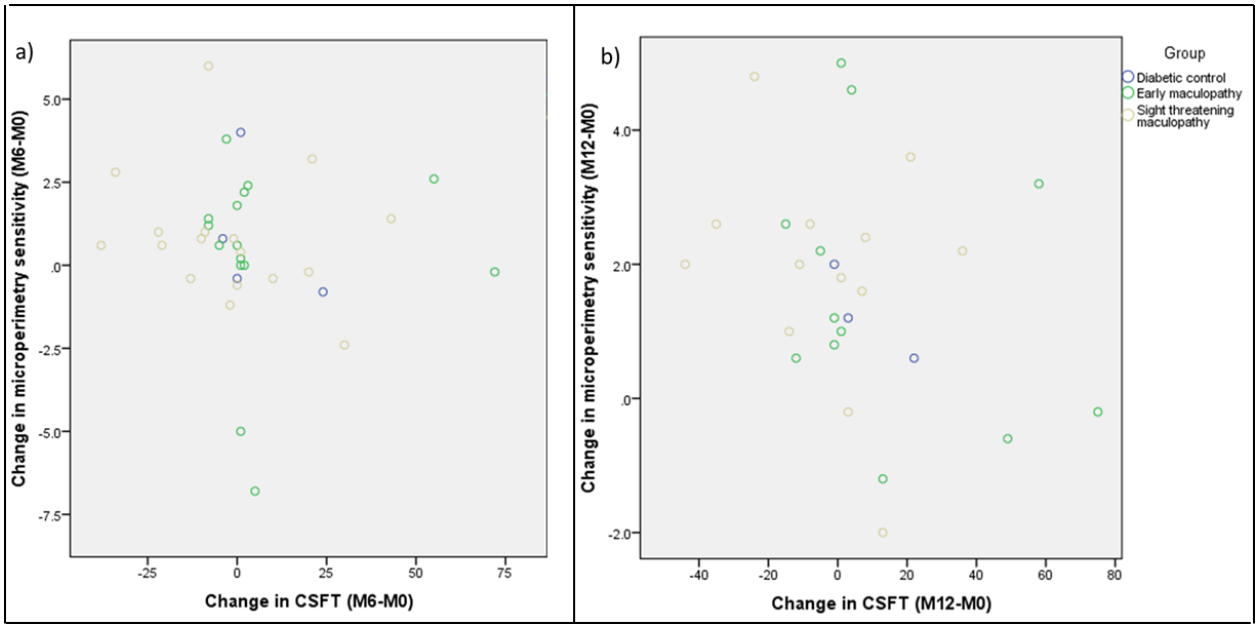
7.4 Correlating central macular function and structure

I analysed the relation between OCT CSFT and central macular function to determine if change in thickness corresponded to change in function. I therefore performed a regression analysis using ANOVA to determine if there was any correlation (*r*) between change in CSFT and central macular function. P<0.05 was deemed significant.

7.4.1 Microperimetry

At both month 6 and month 12 there was no significant correlation between change in MP sensitivity and change in CSFT (p>0.10 for both comparisons) (Figure 7.2 a,b). At month 6 increased CSFT was non-significantly associated with improved MP sensitivity (*r*=0.11, p>0.50). At month 12 the trend was reversed with increasing CSFT non-significantly associated with decreasing MP sensitivity (*r*=-0.28, p>0.10).

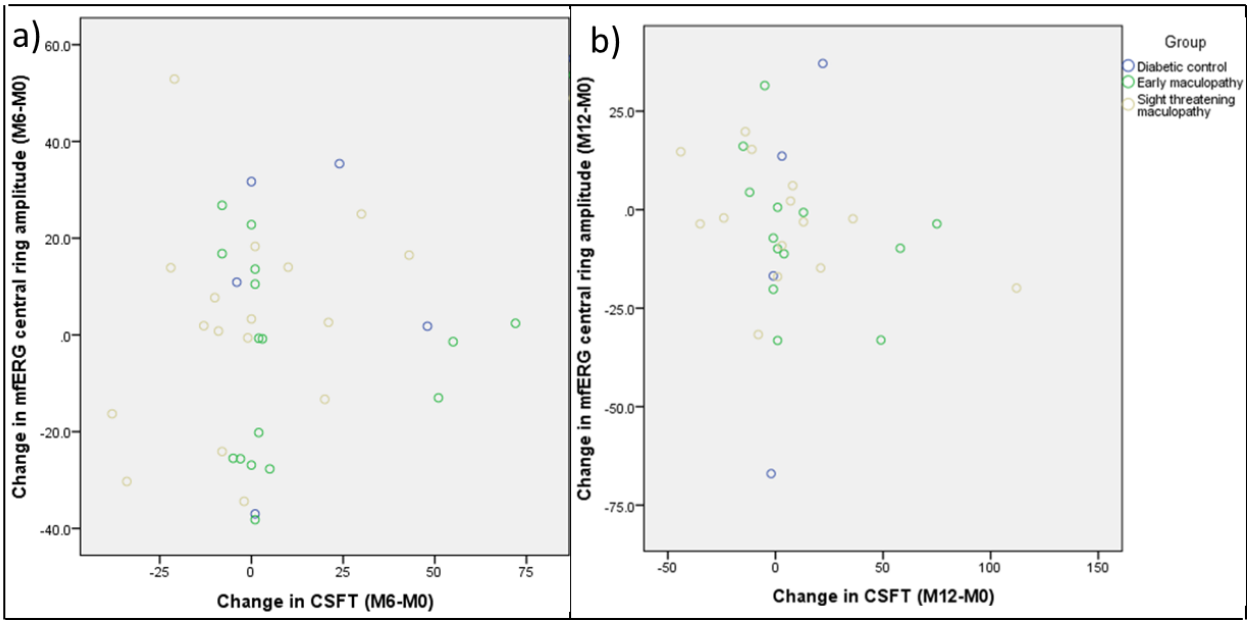
Figure 7.2: Analysis of mean change in CSFT (µm) compared to change in MP central ring sensitivity (dB) at a) month 6 and b) month 12. Data presented is of the group as a whole with the groups represented by different colours: blue = diabetic controls; green = early maculopathy; gold = sight-threatening maculopathy



7.4.2 Multifocal ERG central ring amplitude

At both month 6 and month 12 there was no significant correlation between change in CSFT and mfERG central ring amplitude (p>0.20 for both comparisons) (Figure 7.3 a,b). Non-significant improvement in amplitude was seen with increasing CSFT at both 6 months (r=0.09, p>0.50) and 12 months (*r*=0.21, p>0.20).

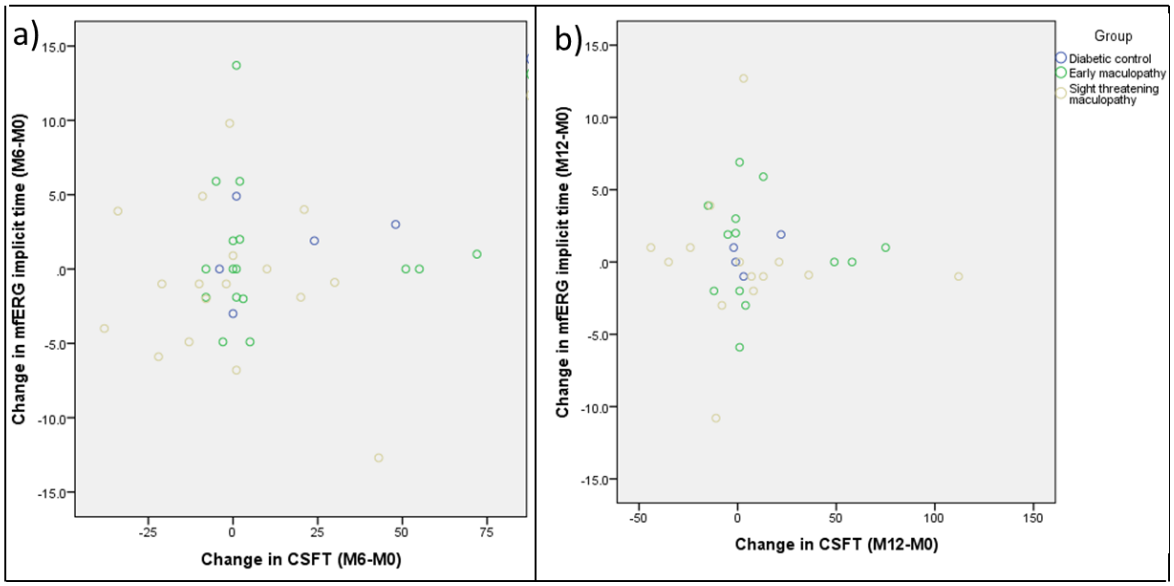
Figure 7.3: Analysis of mean change in CSFT (µm) compared to change in mfERG central ring amplitude (nV/deg^2) at a) month 6 and b) month 12. Data presented is of the group as a whole with the groups represented by different colours: blue = diabetic controls; green = early maculopathy; gold = sight-threatening maculopathy



7.4.3 Multifocal ERG central ring implicit time

There was no significant correlation between change in CSFT and change in mfERG central ring IT at both month 6 (*r*=-0.02, p>0.50) and month12 (*r*=-0.03, p>0.50) (Figure 7.4)

Figure 7.4: Analysis of mean change in CSFT (µm) compared to change in mfERG central ring implicit time (ms) at a) month 6 and b) month 12. Data presented is of the group as a whole with the groups represented by different colours: blue = diabetic controls; green = early maculopathy; gold = sight-threatening maculopathy



7.4.4 Oscillatory potential sum amplitude

There was no significant correlation between change in CSFT and change in OP sum amplitude at both month 6 and month 12 (Figure 7.5). At month 6, there was a non-significant improvement in amplitude with increase in CSFT (r=0.20, p>0.20). At 12 months there was no correlation at all (r=0.00, p>0.50).

7.4.5 Oscillatory potential 1st peak implicit time

There was no significant correlation between changes in CSFT and OP 1st peak IT (Figure 7.6). At month 6 improvement in OP IT was seen with increasing CSFT though this did not reach statistical significance (r=-0.27, p>0.10). A similar trend was seen at month 12 though once again this did not reach statistical significance (r=-0.22, p>0.20).

Figure 7.5: Analysis of mean change in CSFT (µm) compared to change in OP sum amplitude (µV) at a) month 6 and b) month 12. Data presented is of the group as a whole with the groups represented by different colours: blue = diabetic controls; green = early maculopathy; gold = sight-threatening maculopathy

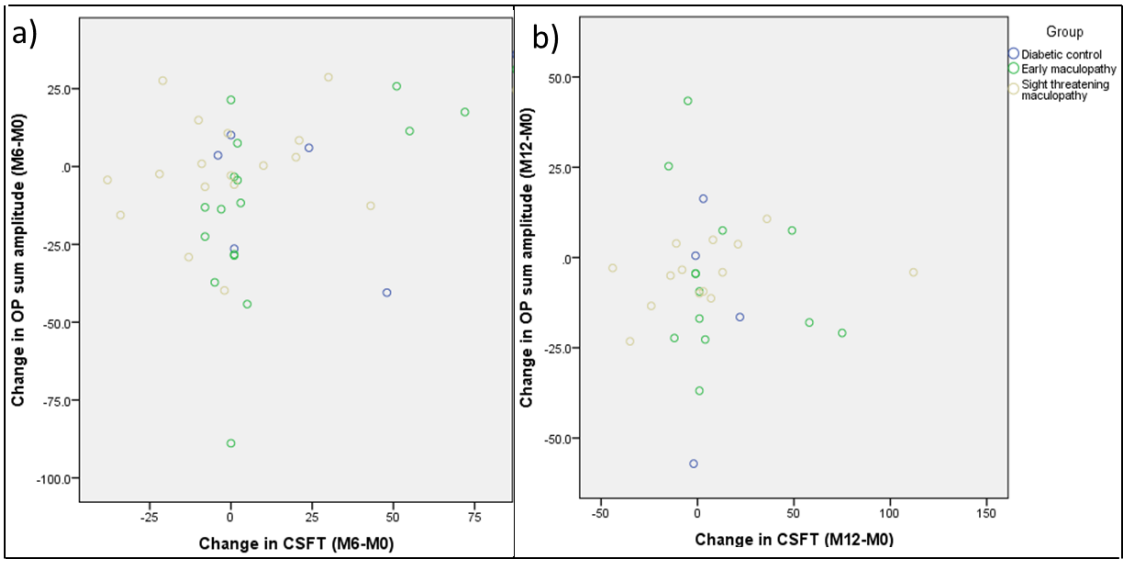
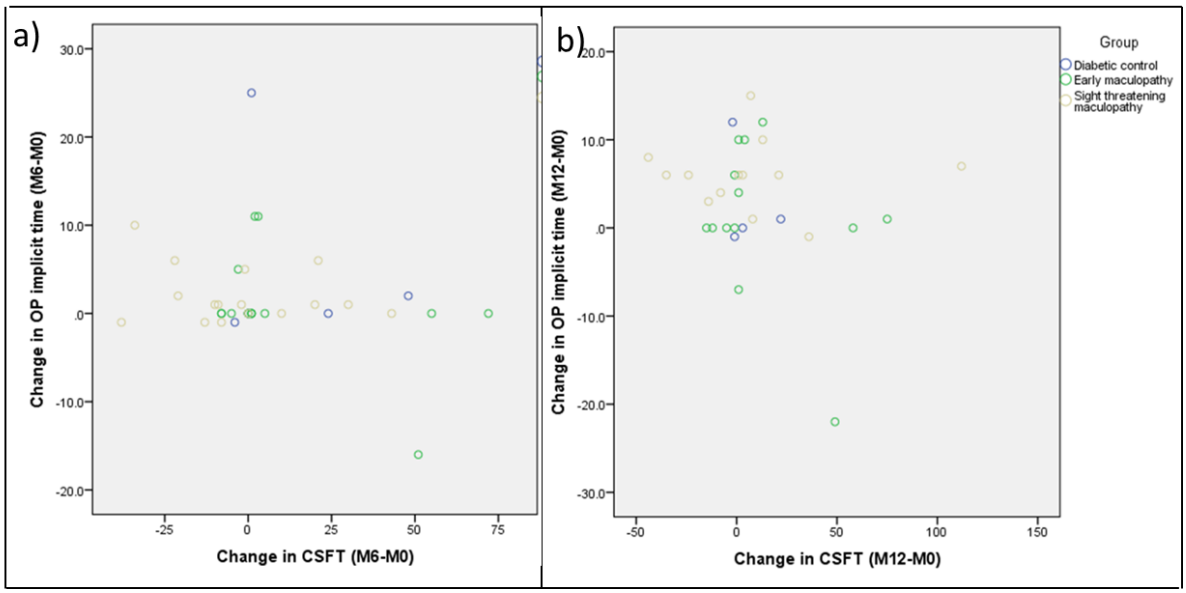


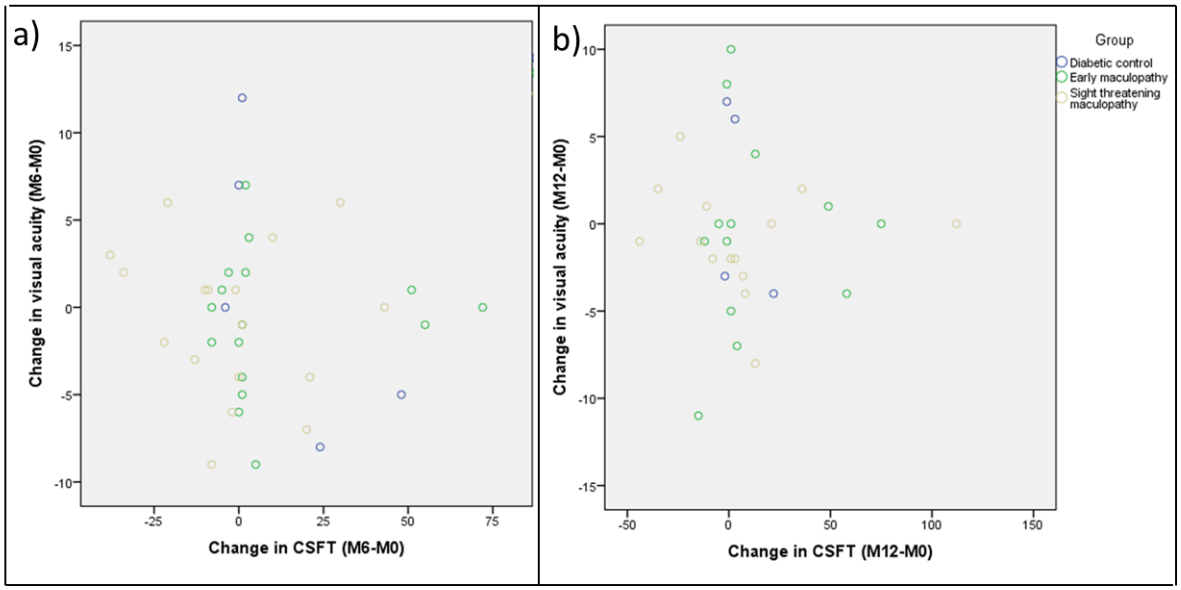
Figure 7.6: Analysis of mean change in CSFT (µm) compared to change in OP 1st peak implicit time (ms) at a) month 6 and b) month 12. Data presented is of the group as a whole with the groups represented by different colours: blue = diabetic controls; green = early maculopathy; gold = sight-threatening maculopathy



7.4.6 Best corrected visual acuity

There was no statistically significant correlation between change in BCVA and change in CSFT at both month 6 and month 12 (Table 7.7). Non-significant improvement in BCVA was seen with increased CSFT at both month 6 (r=0.12, p>0.20) and month 12 (r=0.04, p>0.50)

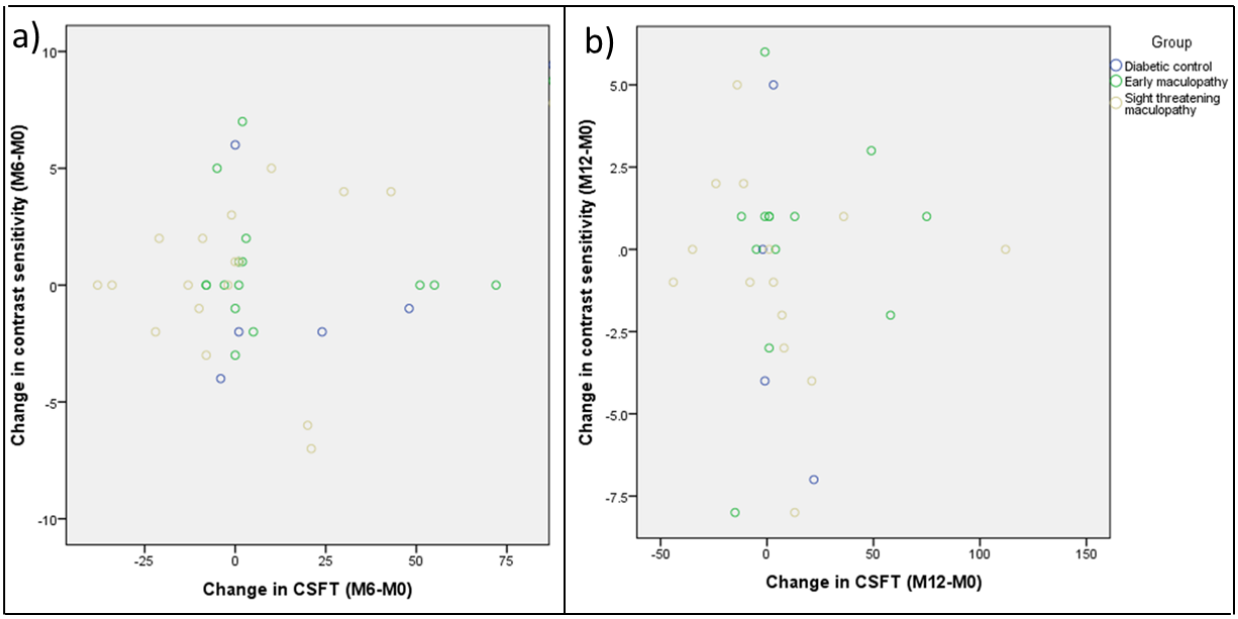
Figure 7.7: Analysis of mean change in CSFT (µm) compared to change in best corrected visual acuity (letters) at a) month 6; and b) month 12. Data presented is of the group as a whole with the groups represented by different colours: blue = diabetic controls; green = early maculopathy; gold = sight-threatening maculopathy



7.4.7 Contrast sensitivity

There was no statistically significant correlation between change in CSFT and change in CS (p>0.50 for both comparisons) (Figure 7.8). There was a non-significant improvement in CS with increasing CSFT at both month 6 (r=0.02, p>0.50) and month 12 (r=0.03, p>0.50).

Figure 7.8: Analysis of mean change in CSFT (µm) compared to change in contrast sensitivity (letters) at a) month 6; and b) month 12. Data presented is of the group as a whole with the groups represented by different colours: blue = diabetic controls; green = early maculopathy; gold = sight-threatening maculopathy



7.5 Analysis of association and prognostic potential of central macular function

In Chapter 6, I described and reported on predictive associations between central macular function and subjects with sight-threatening maculopathy. I used a similar approach to investigate prognostic associations of visual and central macular function could that may predict the requirement of treatment for diabetic maculopathy at both 6 months and 12 months.

Of subjects with DM, 15 underwent treatment prior to the month 6 visit and a further three underwent treatment prior to the month 12 visit. The critical value for month 6 was calculated by determining the lower quartile of visual and macular function of the 15 subjects who underwent treatment plus the 39 subjects who completed month 6; for month 12, the critical value was determined by calculating the lower quartile of the 18 subjects who required treatment plus the 31 subjects

who completed the study. Association was calculated using the Fisher exact test; binomial confidence intervals were calculated using the Clopper-Pearson formula.

The following indices were analysed:

- Association: the significance that a subject with function worse than the critical value will require treatment
- Sensitivity: the percentage of subjects requiring treatment that have function worse than the critical value
- Specificity: the percentage of subjects who do not require treatment have function better than the critical value
- Positive predictive value (PPV): the percentage of subjects with function worse than the critical value who require treatment
- Negative predictive value (NPV): the percentage of subjects with function better than the critical value who do not require treatment

In Tables 7.13 and 7.14 I present my results for month 6 and month 12, respectively.

At month 6, there was a significant association between requirement for treatment and reduced function in BCVA, CS and MP. There was a trend towards reduced function using mfERG central ring amplitude.

For functional measures that showed significant prognostic associations, sensitivity was fair (46.7-66.7%) and specificity was good (87.2-92.3%). There was a fair/moderate PPV (58.3-71.4%) and good NPV (81.0-83.7%).

At month 12, only CS ($p<0.01$) and MP ($p<0.03$) remained significant and there was a trend towards reduced BCVA ($p>0.05$). None of the other investigations showed significance towards requirement for treatment ($p>0.20$ for all comparisons).

For CS and MP, sensitivity was moderate (55.6% and 41.2%, respectively) and specificity was high (90.3% for both). Both PPV and NPV were moderate for both investigations (70.0-77.8 for all comparisons).

Table 7.13: Association of baseline visual and central macular function and subjects who subsequently required treatment for maculopathy by month 6. *p<0.05 deemed significant. amp = amplitude; IT = implicit time; PPV = positive predictive value; NPV = negative predictive value. CI = confidence interval

Investigation	Critical value	Association (p value)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
BCVA (letters)	≤79.0	<0.02*	46.7 (21.3-73.4)	87.2 (72.6-95.7)	58.3 (27.7-84.8)	81.0 (65.9-91.4)
CS (letters)	≤34.0	<0.01*	66.7 (38.4-88.2)	89.7 (75.8-97.1)	71.4 (41.9-91.6)	87.5 (73.2-95.8)
MP (dB)	≤12.6	<0.01*	50.0 (23.0-77.0)	92.3 (79.1-98.4)	70.0 (34.8-93.3)	83.7 (69.3-93.2)
mfERG amp (nV/deg^2)	≤34.1	0.06	42.9 (17.7-71.1)	84.6 (69.5-94.1)	50.0 (21.1-78.9)	80.5 (65.1-91.2)
mfERG IT (ms)	≥41.2	1.00	28.6 (8.4-58.1)	71.8 (55.1-85.0)	27.3 (7.8-55.1)	73.7 (56.9-86.6)
OP sum amp (µV)	≤38.2	0.18	46.2 (19.2-74.9)	74.4 (57.9-87.0)	37.5 (15.2-64.6)	80.6 (64.0-91.8)
OP IT (ms)	≥20.0	0.35	23.1 (5.0-53.8)	89.5 (75.2-97.1)	42.9 (9.9-81.6)	77.3 (62.2-88.5)

Table 7.14: Association of visual and central macular function and subjects who subsequently required treatment for maculopathy by month 12. *p<0.05 deemed significant. amp = amplitude; IT = implicit time; PPV = positive predictive value; NPV = negative predictive value

Investigation	Critical value	Association (p value)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
BCVA (letters)	≤79.0	0.07	38.9 (17.3-64.3)	87.1 (70.2-96.4)	63.6 (30.8-89.1)	71.1 (54.1-84.6)
CS (letters)	≤34.0	<0.01*	55.6 (30.8-78.5)	90.3 (74.3-98.0)	76.9 (46.2-95.0)	77.8 (60.9-89.9)
MP (dB)	≤12.6	<0.03*	41.2 (18.4-67.1)	90.3 (74.3-98.0)	70.0 (34.8-93.3)	73.7 (56.9-86.6)
mfERG amp (nV/deg^2)	≤34.1	0.30	35.3 (14.2-61.7)	80.6 (62.5-92.6)	50.0 (21.1-78.9)	69.4 (51.9-83.7)
mfERG IT (ms)	≥41.2	1.00	29.4 (10.3-56.0)	71.0 (52.0-85.8)	35.7 (12.8-64.9)	64.7 (46.5-80.3)
OP sum amp (µV)	≤38.2	0.35	43.8 (19.8-70.1)	71.0 (52.0-85.8)	43.8 (19.8-70.1)	71.0 (52.0-85.8)
OP IT (ms)	≥20.0	0.22	25.0 (7.3-52.4)	90.0 (73.5-97.9)	57.1 (18.4-90.1)	69.2 (52.4-83.0)

There have been no studies that have assessed the role of investigations of visual and central macular function in predicting the requirement for treatment as determined by a clinician. In my study, CS and MP central ring sensitivity may identify subjects who require treatment of their maculopathy at within both 6 and 12 months, whilst BCVA may aid at 6 months. The other investigations of macular function have not been shown to be beneficial to identify those 'at risk'. These findings are a reflection of current clinical practice in which reduction of visual

acuity often prompts clinical treatment. The lack of significance with mfERG and OP may be due to the wide variations in amplitude and IT as described in Chapter 6. It is probable that there is a critical point in neuronal function beyond which microvasculopathy, and subsequently sight-threatening disease, develops. This may explain why subjects with early maculopathy showed a reduction in function in MP central ring sensitivity and mfERG amplitude (see Chapter 6) and all but one did not require treatment.

As with the analysis of association for diagnosing sight-threatening maculopathy in Chapter 6, these results should be treated with caution. This study was not powered for such an analysis. In addition the short follow-up time and the use of local study protocols mean that the results may not applicable to determine predictability in the long-term and in a different cohort of patients, where the critical value may be different.

7.5 Discussion

7.5.1 Key results

In this chapter the following statistically significant changes in visual function were seen in the group as a whole:

- a 11% increase in MP central ring sensitivity at 12 months

The following trends were seen in the group as a whole:

- a 4% increase in MP central ring sensitivity at 6 months
- a 11% decrease in OP sum amplitude at 12 months

Sub-group analysis revealed the following significant changes in visual function:

- a 13% increase in MP central ring sensitivity in the sight-threatening maculopathy group at 12 months

Sub-group analysis revealed the following trends:

- an increase in MP central ring sensitivity in sight-threatening maculopathy group at 6 months

- an increase in MP central ring sensitivity in early maculopathy group at 12 months
- a decrease in mfERG central ring amplitude across all groups at 12 months
- a decrease in OP sum amplitude across all groups at 6 and 12 months

Analysis of structure on OCT revealed no specific trend at 6 months, but there was an increase in thickness across all groups at 12 months. Change in CSFT was not significantly correlated to change in visual function. However increase in CSFT appeared to be non-significantly associated with improvement in visual function across all investigations apart from MP and OP sum amplitude at 12 months.

CS and MP central ring sensitivity appeared to be prognostic for the requirement of treatment for maculopathy at both month 6 and month 12. BCVA appeared to be prognostic for requirement of treatment at month 6 but not month 12.

7.5.2 Microperimetry

In my study, there was an overall significant improvement in sensitivity of 1.7 dB ($p<0.01$) at month 12. There was an improvement in sensitivity at month 6, though this did not reach statistical significance (mean 0.6 dB, $p<0.02$). Sub analysis revealed that at month 12 there was a significant improvement in the sight-threatening maculopathy group (mean 1.9 dB, $p<0.01$) and a trend towards improved sensitivity in the early maculopathy group (mean 1.6 dB, $p<0.02$).

There has been one study that has assessed change in foveal sensitivity in subjects with DM. In a longitudinal study of 2 years, 25 subjects with DM and 20 healthy controls were assessed using BCVA, OCT and the Rarebit Fovea Test (RFT).[215] The RFT records the success of detecting stimuli when fixating on a central fixation cross and is recorded as the mean hit rate (MHR). In this study MHR below 97% was recorded as subnormal and a test area that covered the central 4^{o} of the macula was used. The authors reported that significantly more subjects with DM (12/25) stayed or became subnormal compared to healthy controls (2/20, $p<0.01$). One subject had mild maculopathy at baseline which had not altered clinically but there was a decrease in MHR and thinning on OCT. There was no significant difference in VA or OCT CSFT during the study. The authors also reported change in retinopathy

at four to six years from baseline with a third developing worsening; however there was no correlation to RFT at baseline.

In my study subjects were followed up for only one year and we only analysed the central macula. The improvement in sensitivity probably reflects a learning effect and so a longer follow up would provide a more accurate reflection of longitudinal changes in retinal function. Also assessment of just the central macula may not accurately reflect changes in neuronal function, and analysis of the macula as a whole could be more beneficial.

7.5.3 Multifocal electroretinogram

In my study there was no significant difference in either amplitude or IT at both month 6 and month 12 for subjects with DM. There was an overall worsening in amplitude (mean -5.0 nV/deg^2, p>0.10) at month 12 with all groups showing non-significant worsening. There was a slight prolongation of IT at 12 month (mean 0.4ms, p>0.50) though there was no specific pattern across the groups.

Pescosolido et al (2015) stated that mfERG is beneficial in assessing early disease but is not suitable for follow-up studies, especially after treatment.[216] This is due to wide inter- and intra-subject variability in mfERG, especially amplitude. They also state that structural assessments such as OCT and FA offer a more objective analysis of maculopathy progression. Other studies have stated that mfERG findings do not correlate with changes in retinal structure after treatment.[217,218]

In my study I assessed change in central ring amplitude and IT over a year. However published literature has focused on development of vasculopathy in areas of reduced macular function and have not reported changes in actual values of these investigations.[205,219]

In a study of 11 subjects with DR and 11 subjects without DR, mfERG IT was assessed to predict onset of development of DR.[220] The authors utilised 103 hexagons which were assessed for presence or absence of retinopathy and a Z-score was determined for each hexagon. The authors reported that, over 1 year,

none of the subjects without DR developed DR and mfERG IT did not alter significantly. The authors concluded that retinal function remains stable over 1 year.

In a further study by Han et al (2004)[200] 12 subjects with NPDR and 16 subjects with no DR were assessed over 1 year for development of new retinopathy. After 1 year, 11/12 of the NPDR and 1/16 of the no DR groups developed new retinopathy. The authors performed a multivariate analysis using mfERG IT, duration of DM, presence of retinopathy and blood glucose level at baseline. The authors reported that this multivariate model had a sensitivity of 86% and specificity of 84%. However there was no report on development of maculopathy.

Harrison et al (2011) assessed development of DR in 78 eyes of subjects with no DR over a mean follow up time of 3 years.[221] Using a 103 hexagon array, the authors calculated 35 retinal zones and assigned a *Z*-score based on normative data. The authors reported that increased IT and reduced amplitude were more likely to develop macular oedema. The same group also reported that for every 0.9 ms increase in IT there was a 16% increased risk of developing retinopathy.[147] The authors concluded that mfERG IT, after adjustment for confounders, is predictive for development of DR. However these findings were based on all macular loci whilst I focused on just the central macula. There was no quoted risk of developing macular oedema.

Adams & Bearse Jr (2012) analysed 46 eyes of 23 patients over 2 years.[222] They reported that only 5.2% of retinal zones and 35% of eyes developed DMO. Over half of these eyes developed CSMO. MfERG amplitude, IT, systolic blood pressure and gender were predictive for onset of DMO. Once again this study reported on all 35 retinal zones of a 103 hexagon grid rather than just the central macula.

In summary, mfERG amplitude in the central macula appeared to decrease over 12 months in subjects with DM and there was a slight prolongation in IT. This may reflect ongoing neuronal disruption associated with DM though the significance of rate of such loss is yet to be established. Longer studies with a larger cohort are required to determine whether subjects with an increased rate of function loss are more likely to develop maculopathy.

7.5.4 Oscillatory potentials

In my study, there was a trend towards an 11% reduction in OP sum amplitude over 1 year (mean -6.2 μV, p=0.03). However there was no trend seen with IT. There was a 9-10% reduction in amplitude at 12 months for both the early and sight-threatening maculopathy groups, though this was not significant (p>0.05). This suggests that OP function may decrease in the presence of maculopathy regardless of severity of disease. There are no previously published studies with which to compare these results.

Like longitudinal studies of mfERG, studies in OP have been used to predict development of retinopathy. Vadala et al (2002) assessed OP amplitude in 80 subjects with insulin-dependent DM and no DR over a period of 10 years.[160] The authors reported that 35% of subjects developed DR. Of those who developed DR, less than half (46%) of subjects developed reduction in OP amplitude to subnormal levels; however decrease in OP amplitude was also noted in 25% of subjects in whom the fundi remained normal. Of those who developed retinopathy, 61% developed subnormal levels of amplitude prior to development of vasculopathy. However 39% developed reduced amplitudes concomitantly or after development of vasculopathy. The authors concluded that OP amplitude on its own may not predict onset of DR development.

In summary, OP sum amplitude appears to decrease longitudinally in subjects with DM. With a similar finding in mfERG amplitude, this suggests continuing decline in neuronal function in the inner retina. It is possible that longitudinal assessment of both mfERG and OP amplitudes may aid in the identification of subjects at risk of developing diabetic maculopathy by determining rate of neuronal dysfunction.

7.5.5 Contrast sensitivity

In my study there was a clinically insignificant decrease in CS at 12 months (mean -0.5 letters, p>0.50). There was no specific trend in subjects with maculopathy. There is no published literature with which to compare these findings. Therefore no specific conclusions can be drawn on the benefit of longitudinal assessment of CS in my cohort of subjects.

7.5.6 Subjects who received treatment

Of the 87 subjects who completed the baseline visit, 15 (17%) underwent treatment for their maculopathy prior to the 6 month visit. A further three subjects underwent treatment for their maculopathy. Those who underwent treatment were found to have a higher HbA1c than those who had withdrawn from the study and those who had completed the study. In addition the treated group had worse BCVA and contrast sensitivity.

The Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) has been described in Chapter 3. Important findings in the study included elevated HbA1c was associated with an increased risk of developing diabetic macular oedema and CSMO.[69] The ACCORD Eye study also reported reduction in progression of DR in subjects treated with intensive glycaemic control.[122] This fits in with our finding that those who received treatment had a greater HbA1c.

There was no difference in serum cholesterol between those who were treated and those who were not treated. Zander et al (2000) reported that subjects with type 1 DM, but not type 2, and maculopathy had significantly higher cholesterol levels.[129] The Early Treatment Diabetic Retinopathy Study (ETDRS) reported that subjects with elevated cholesterol were more likely to develop hard exudates and significant visual loss.[119] The ACCORD Eye study and the FIELD study reported that subjects who received a lipid lowering agent demonstrated decreased rate of progression and reduced requirement for treatment of their maculopathy.[122,123] However the United Kingdom Prospective Diabetes Study (UKPDS) demonstrated minimal benefit in development of microvascular complications following treatment of elevated serum cholesterol levels.[125,134]

As with serum cholesterol, there was no significant difference in systolic and diastolic BP between those were treated and those who did not receive treatment. The UKPDS demonstrated that reduction in BP was associated with a reduced requirement for laser for maculopathy.[125,134] However the ACCORD eye study demonstrated no significant difference in progression of retinopathy with intensive BP control.[122]

In my study, subjects who received treatment demonstrated no significant difference in BP or serum cholesterol. This could be because these subjects had worse systemic disease prior to entering the study and so had already received systemic treatment. All but one subject who received treatment had sight-threatening maculopathy at baseline. In addition our baseline systemic findings may not accurately reflect prior systemic control.

7.6 Limitations of the study

Of the 89 subjects with DM recruited to the study, 61 (69%) were invited to follow up. Of these only 39 completed the 6 month visit and 31 completed the 12 month visit. The analyses performed above show that there was no significant difference in systemic risk factors between subjects who withdrew and those who completed the study at both 6 months and 12 months. This would have minimised any bias that may have been introduced by subjects being withdrawn. However the low number of subjects within each group makes comparisons within each group difficult. I will explore the reasons for low follow-up in Chapter 8 and how this may be addressed.

Due to the short duration of follow up (12 months), the discrepancies in results of macular function may reflect the variations seen in the fluctuating appearance of retinal changes in diabetic retinopathy and maculopathy. A study of longer duration may better elucidate changes in function as more permanent microscopic and macroscopic changes in retinal structure take hold.

No healthy controls were invited to take part in the longitudinal study. Therefore there is no comparison to determine whether longitudinal changes in function were due to DM alone. In future studies, the longitudinal assessment of healthy controls will be required to determine normative data for change in macular function longitudinally. This normative data could then be used to determine significance of rate in change in function in DM and other retinal conditions.

In my study I focused on assessment of the central macula. My aim was to determine central macula function to identify risk of developing sight-threatening maculopathy. As a result I did not analyse a large portion of the macula. Analysing

the whole macula may provide a more comprehensive view of function, or dysfunction, in disease.

There is wide variability in mfERG and OP recordings. Therefore, in small groups, any outliers are likely to make a significant change to mean values. For example, in the OP implicit time recordings, the early maculopathy group showed improvement at 12 months. That was because one subject showed an estimated improvement of 8 ms compared to baseline.

A recent study of 317 eyes of subjects with type 2 DM reported increased thickness of CSFT was significantly associated with an increased risk of developing clinical macular oedema.[223] In addition, involvement of the inner and outer rings was associated with an increased risk of developing clinical macular oedema. The authors concluded that combining thickness of the outer and inner rings would offer a better characterisation of DMO. I did not analyse OCT CSFT with regards to risk of developing maculopathy. However, it may be likely that combining assessment of structure and function may offer a comprehensive way of predicting DMO formation.

7.7 Summary

In this chapter I have demonstrated that MP sensitivity appeared to improve longitudinally in the first year, especially in the sight-threatening maculopathy group. There appeared to be a trend towards reduction in mfERG and OP amplitude as this was seen in all groups, but no change in BCVA or CS, indicating possible progressive reduction in sub-clinical retinal function. The significance of rate of decrease in function is yet to be established. There does not appear to be any correlation between change in CSFT and central macular function. This may be due to different unknown mechanisms in action in which some subjects develop thinning of the retina whilst others develop thickening. Reduced function in MP retinal sensitivity and CS appear to be associated with need for treatment; this may reflect current clinical practice of treating subjects with visual dysfunction.

In Chapter 8 I will explore the role of investigations of macular function in the assessment, and their potential roles in their identification, of those at risk of developing diabetic maculopathy.

Chapter 8 Discussion

Diabetes mellitus (DM) is a growing epidemic with diabetic eye disease, especially diabetic maculopathy, being a significant contributor to visual impairment. Screening programmes based around visible clinical findings have reduced, but not eliminated, the risk of developing visual impairment. Neuronal dysfunction of the macula in DM has been shown to occur prior to the development of microvasculopathy and has been studied in diabetic retinopathy (DR). My aim was to assess neuronal function, and dysfunction, to determine the role functional assessment of the central macula could play in determining severity of diabetic maculopathy. In this chapter I explore the key results that I have described in Chapters 6 and 7 and I discuss the possible contributions of microperimetry (MP), multifocal electroretinography (mfERG), oscillatory potentials (OP) and contrast sensitivity (CS) to the management of diabetic maculopathy.

8.1 Severity of maculopathy

In my study, subjects with sight-threatening maculopathy showed significant reductions in central macular function across all parameters (mean visual acuity (VA), CS, MP, mfERG amplitude and implicit time, and OP). These associations were the main aim of my thesis and the study design was powered accordingly. I believe therefore that these biomarkers are valid for this population of patients. Subjects with early maculopathy showed trend reduction in function in some parameters (VA, CS, MP and mfERG amplitude).

Measures of macular function other than BCVA are attractive as diagnostic biomarkers in early and moderate sight threatening maculopathy. In order for associations to be credible as diagnostic markers however a number of conditions need to be met including sufficiently high sensitivity, specificity, positive and negative predictive value and external validation.[224] The sensitivities in my study were only moderate which can be explained by either them not having strong diagnostic potential or by the limited study size. Nonetheless my findings are biologically plausible and strengthen the research area.

The potential of the measures of macular function as diagnostic biomarkers could be strengthened with higher critical values. There may be a critical value for each type of function beyond which the likelihood of a subject having, or developing, sight-threatening maculopathy substantially increases. I selected a critical value based upon the lower quartile of all subjects with DM. The reason I used this cut-off was because there were no previous studies upon which to base a critical value. If I used a lower cut-off, for example 5%, this would have considerably reduced sensitivity and negative predictive value of each investigation. An alternative would be to base the critical value upon the healthy controls rather than subjects with DM. Critical values could be set at 10%, 20% and 50%. Sensitivity and specificity could then be calculated and the critical value associated with the highest sensitivity and specificity could be used for future studies. Another approach would be to set sensitivity to 80% and then calculate the critical value that leads to such sensitivity levels and calculate specificity and positive and negative predictive based on that. In my study I did not analyse sensitivity and specificity with outcomes adjusted for age; this would need to be performed in future studies.

By identifying these critical values for each measure, it may be possible to stratify the risk of a subject developing diabetic macular oedema and/or ischaemic maculopathy. This risk stratification could have implications on follow-up, initiation of ophthalmic treatment and systemic treatment and form part of the individualised patient care which has become the new aim in healthcare.

Another way of looking at the outcomes of each investigation of this study is the data presented in the boxplots in Chapter 6. In CS, MP and mfERG central ring amplitude and implicit time, one can see a small deterioration of function from diabetic controls to early maculopathy and then a steeper drop in function to sight-threatening maculopathy. There may be a critical point at which a certain percentage of neurons are lost beyond which function deteriorates substantially. Through my study I have been unable to detect this critical point, though it is likely to be within the range of outcomes of subjects with early maculopathy as the greatest deterioration was between this and the sight-threatening maculopathy groups. In my study greatest mean reduction in function in the sight-threatening

maculopathy group was seen in mfERG amplitude compared to MP. Therefore, it is likely that mfERG amplitude could be more sensitive to picking up earlier deterioration in function and thus be useful in the detection of this critical point.

8.2 Early maculopathy

In my study, subjects with early maculopathy showed trends towards reduced function in the central macula for BCVA, CS, MP and mfERG amplitude compared to healthy controls. However these differences were not statistically significant and so it is difficult to determine the benefit of assessment of central macular function in early disease.

Other studies have reported reduced function in early or no disease. However these studies have predominantly focused on assessment of the whole macula rather than the central macula. This suggests that in early disease analysis of the whole macula should be performed using functional assessments. Using a similar method described above, critical values based on outcomes of the whole macula could be determined. A threshold at the worst quartile (75%) could allow subjects with function worse than the critical value to undergo analysis of central macular function to determine severity of maculopathy. This could lead to disease profiling or stratification to identify those at risk of developing visual impairment.

Eventually, using the critical values, a chart or algorithm could be produced such that functional and structural outcomes could be combined with systemic risk factors to provide patients with an accurate risk of developing maculopathy and subsequently require treatment.

8.3 Amplitude vs implicit time

Amplitude represents strength of response to a stimulus while IT represents efficiency of response. In my study, amplitude in both mfERG and OP appeared to be more affected than IT in maculopathy. As described in Chapter 3, several other studies have reported IT to be more sensitive than amplitude especially for mfERG. This discrepancy is likely due to differences in study protocol. As reported in Chapter 5 my study used a 19 hexagon array whilst most other previously published

studies used a 61 or 103 hexagon array to cover roughly the same area. Therefore each hexagon in my study covered a larger area of the macula and therefore generated greater amplitude compared to other studies. In addition, the central macula generates the greatest amplitude compared to other parts of the macula. Therefore, by focussing on the central macula, reduction in amplitude in disease is likely to be more meaningful using the 19 hexagon array.

In my study mfERG IT appeared to be less sensitive than amplitude in assessing diabetic maculopathy and test of association was non-significant in the diagnosis of sight-threatening maculopathy. This is most likely due to my study protocol in which a stimulus is presented every 83.3 ms compared to 16.7 ms in other protocols. This delay in stimulus presentation allows the neurons time to repolarise to resting state and be ready for the next stimulus. Therefore, in early disease, any slight decrease in function may not be detected. Therefore, if decrease in IT is noted then there must be sufficient disease.

However, these differences may also represent different structures of the retina being affected. Subjects with DMO show a wide variation of structural changes. It is therefore possible that different neuronal cells are affected and so there could be a variation in neuronal dysfunction between subjects with similar clinical diagnosis. By combining different assessments of macular structure and function, it may be possible to determine whether a subject has disease within a specific retinal layer or whether the whole retina is diseased.

8.4 Oscillatory potentials

OP sum amplitude was significantly reduced in sight-threatening maculopathy but not early maculopathy; for both groups there was no significant trend with OP IT. The reduction in function was less with OP than with mfERG. This may be because OP is an assessment of global retinal function. Therefore there would need to be significant retinal disease before reduced function is detected. This suggests OP would not be an adequate investigative tool for diabetic maculopathy. More recently, multifocal OP has been performed and shown to be useful in assessing

macular disease and may be an avenue to explore further in the analysis of diabetic maculopathy.

8.5 Optical coherence tomography

OCT has become an integral part of the assessment of diabetic maculopathy and monitoring response to treatment, often being used as a primary outcome. In my study, there was significant thickening in central subfield thickness (CSFT) in the sight-threatening maculopathy which is to be expected. There was no significant difference between healthy controls, diabetic controls and subjects with early maculopathy. This suggests that OCT CSFT is useful in more severe disease but not for early disease. However I only assessed the CSFT. Subjects with early maculopathy had features that did not meet the criteria for clinically significant macular oedema and so would have been outside the central subfield. This may explain why these subjects showed no significant changes.

I did not correlate retinal function to CSFT, but there was no correlation between change in retinal function and change in CSFT in longitudinal analysis. Published studies have reported changes in OCT thickness in relation to maculopathy. However there appears to be some debate around what is abnormal. Some studies report retinal thinning to be indicative of DR whilst others have reported retinal thickening to be more indicative. The former is meant to represent neuronal loss whilst the latter represents microvascular leakage. Comparison of retinal function to retinal thickness has previously suggested that reduced function is associated with retinal thinning. In addition increased retinal thickness has been shown to be predictive of developing maculopathy. I recommend that both OCT and assessments of retinal function be used in analysis of maculopathy though more research is required in the correlation of function to thickness.

8.6 Predicting disease progression

Unfortunately my study showed no consistent trend in change in function over 12 months with an improvement in MP but worsening of OP. This may be due to the

intra-subject variability in amplitude and IT that has been reported previously. Therefore change in function longitudinally may not be a useful approach.

However there was an association between BCVA, CS and MP and requirement for treatment at 6 months, and between CS and MP and requirement for treatment at 12 months. These outcomes reflect current clinical practice. Using the method previously described to determine critical values of each investigation, it was possible to determine likelihood of a subject requiring treatment over a set period of time. For example, in my study subjects with CS ≤34.0 letters at baseline had a 76.9% likelihood of requiring treatment within 12 months.

By altering the critical value using the method described above it may be possible to identify likelihood of requiring treatment based upon mfERG amplitude over 12 months. However neuronal dysfunction is reported to appear before microvascular disease. Therefore, in my study, neuronal dysfunction was likely to be already established and so association was not significant. A higher critical value, for example 50% quartile, would be required and the subjects studied for longer to determine whether mfERG is useful for identifying likelihood of requiring treatment.

8.7 Novel clinical endpoints

The scientific community and the pharmaceutical industry are keen to develop new biomarkers and clinical endpoints as surrogates and/or predictors of vision impairment providing a more accurate and repeatable assessment of retinal function. Interventional studies in diabetic maculopathy currently use best corrected visual acuity (BCVA) as the primary outcome measure, driven primarily by the requirements of the regulatory agencies, especially the FDA. This reliance on BCVA to demonstrate clinical efficacy has hampered the development of several therapeutic options with notable examples of failed phase 3 studies from the DIRECT study and the PKCbeta therapy programme. BCVA is subjective and has reproducibility in various studies at best of +5.5 letters, and more typically of around 10 letters.

In my study, functional assessments appeared to be more severely affected in early/ moderate disease, even in subjects with unaffected BCVA. These assessments may offer a more objective measure of treatment response; the EUROCONDOR study has used mfERG as a primary efficacy measure rather than BCVA. Future studies could assess why some subjects respond well to treatment while others show no significant benefit. It may be possible that subjects who respond inadequately to treatment have severe neuronal dysfunction; therefore any further treatment may be unlikely to offer significant benefit.

Preclinical detection of disease does carry certain disadvantages. There are likely to be false positives resulting in more referrals to specialist services, and so more demand on resources. The associated costs of increased testing may divert funding or resources from other clinical services; though it could be argued that there may be cost-savings through the potential prevention of significant visual impairment. Some patients with DM may undergo more frequent clinical appointments unnecessarily. It is likely that those with minimally reduced function may be subjected to unnecessary investigations and even treatments. False negatives may result in patients at risk of developing visual impairment being missed or not assessed appropriately.

8.8 Multimodal imaging

For my study I developed a method of comparing structural and functional assessments. Due to the use of different imaging and data recording platforms this was challenging. However such a method is required to obtain an accurate comparison between structure and function. This allows for interpretation of specific retinal loci to develop a more comprehensive picture of disease and monitor changes across each investigation.

One disadvantage of combining several investigations is the extensive volume of data generated. This data can be overwhelming and therefore difficult to interpret. I focused on the central macula as I wanted to assess function in the region that is predominantly associated with visual function. As can be seen in Chapter 5, the MP grid was designed such that stimuli could be localised to specific hexagons of mfERG

and quadrants of OCT. It would therefore be possible to assess part or whole of the macula. However this time consuming process may be impractical in clinical practice using current technology. For an investigation to be clinically useful it has to have an easily generated outcome. One recommendation could be to assess part of the macula, for example the nasal macula in the first instance. The nasal macula has previously been shown to be susceptible in early diabetic retinopathy. Combining outcomes form the central and nasal macula may provide more data on macular function than just the central macula alone. Another concern of using larger amounts of data is the risk of dilution of outcomes. If a large area of the macula is analysed simultaneously, then any normal functioning retina included in the analysis would minimise the effect seen by the abnormal retina. Therefore subjects with abnormal central macular function may be mislabelled as normal.

8.9 Recruitment and follow-up

One limitation of my study was the time taken to recruit subjects to the study and the subsequent drop out during follow-up. The time taken to recruit meant that fewer subjects were invited to follow up and this weakened the longitudinal results. It also meant that follow up was limited to one year during analysis.

I pre-screened over 280 subjects for this study. However many potential subjects declined to take part, citing multiple appointments, time constraints and ill-health as the main reasons. It is important to remember that DM is a systemic disorder that can be life-consuming for patients and carers. Therefore any study which requires multiple, long appointments may not appeal to the very people whom the study would offer most benefit.

Once recruited, many subjects dropped out for similar reasons. To encourage continued participation I organised all investigations to be performed in one day and provided flexibility for study visits, such as allowing subjects to arrive later in the morning.

Despite my best efforts, each study visit took a minimum of five hours, and usually took as long as six to seven hours. I would recommend that any study should

consider a maximum of four hours to avoid participant fatigue and reduce dropout rates. Ideally, if the study visit could be combined with a clinic visit then that would most likely encourage better participation. Limiting the number of investigations and using a shortened protocol would also help to speed up the day. Based on my results I would recommend contrast sensitivity, microperimetry and multifocal electroretinography to be incorporated within clinical assessment of diabetic maculopathy along with VA and OCT. In my experience through this study this was achievable within four hours though it would require someone to co-ordinate the process and ensure maximal utilisation of the investigations.

It is still to be established how frequently these tests should be performed, though I would suspect no more than annually in clinical practice. As experience and understanding with these investigations improve the frequency of these tests could be altered depending on severity of ophthalmic and systemic disease.

8.10 Concluding remarks and future studies

Assessments of neuronal function, such as contrast sensitivity, multifocal electroretinography, microperimetry, and oscillatory potential, offer the clinician added information on severity of diabetic maculopathy, especially in the presence of central macular ischaemia. The outcomes of my study demonstrate a reduction in central macular function in sight-threatening maculopathy across all parameters and a trend towards reduced function in some parameters in presence of early maculopathy which is biologically plausible. Microperimetry and mfERG appear to be more sensitive in early maculopathy than OP. These findings add strength to existing data on mfERG and MP as well as provide novel findings of OP and CS in diabetic maculopathy. Exploratory analysis of diagnostic potential adds further strength to the role of tests of functional assessment as potential biomarkers and provides as an alternative to visual acuity for maculopathy analysis. I believe that my data, in conjunction with other published data, should allow for the design of clinical trials in which functional assessments could be used as primary outcomes to study disease progression or effect of an intervention.

Of the functional biomarkers assessed, I feel that MP and mfERG amplitude offer the best potential for diagnosis of diabetic maculopathy. MfERG is an objective assessment and so may offer more reliable recordings; however it requires greater resources (equipment, time, clinic space and personnel) than MP and so would not be practical in a clinical setting. MP is easier to perform and analyse and so could be more easily incorporated into clinical practice. In current clinical practice, MP could potentially offer further information in determining the need for treatment. However, this is a reflection of clinical judgment being based upon treatment of reduced visual function. MfERG and OP amplitude may potentially provide further information on sub-clinical progression and therefore identify need for treatment prior to development of visual impairment. However further studies are required to determine the difference between physiological and pathological change in retinal function longitudinally.

My study is limited by exclusion of subjects with DR but no diabetic maculopathy. This group was excluded as our aim was to assess macular function with respect to diabetic maculopathy, rather than retinopathy, and to limit type 1 errors that may be seen with having a larger dataset. In addition I focused on only the central macula and therefore excluded a substantial proportion of the macula in my analysis. Future studies should include subjects with DR but no diabetic maculopathy to determine effect of maculopathy itself on macular function. Analysis of the whole macula, and not just the central macula, should be performed to determine whether the extra information generated provides a more significant assessment of diabetic maculopathy. A longitudinal study of healthy controls should also be performed to develop normative data on change in function with which to compare.

A study with a larger cohort and use of different thresholds to determine the critical values for each investigation may aid in risk stratification. I believe this is where assessments of macular function would offer most advantage in the management of diabetic maculopathy.

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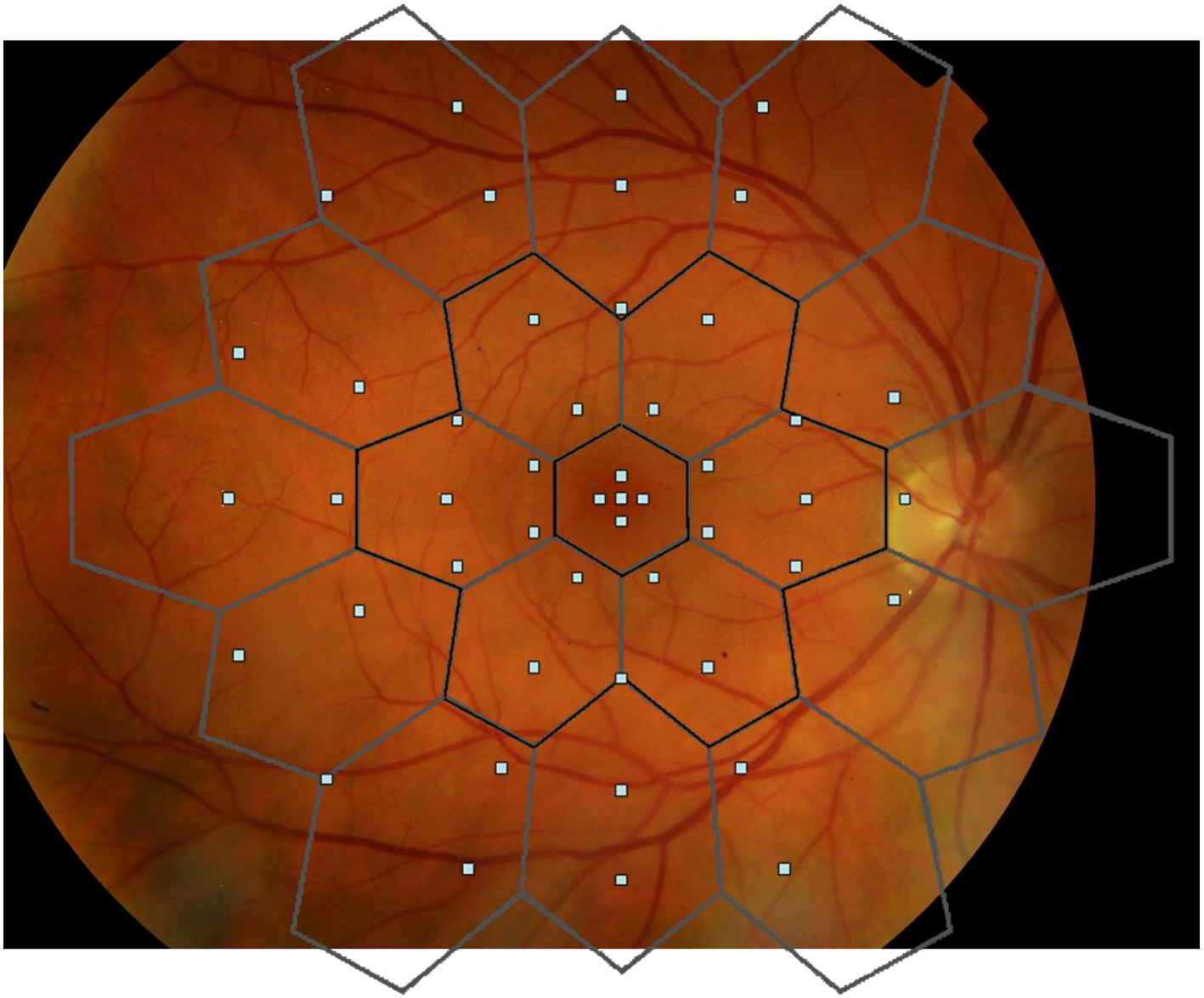
Appendix

Appendix 1 – Combined images of microperimetry and multifocal electroretinogram

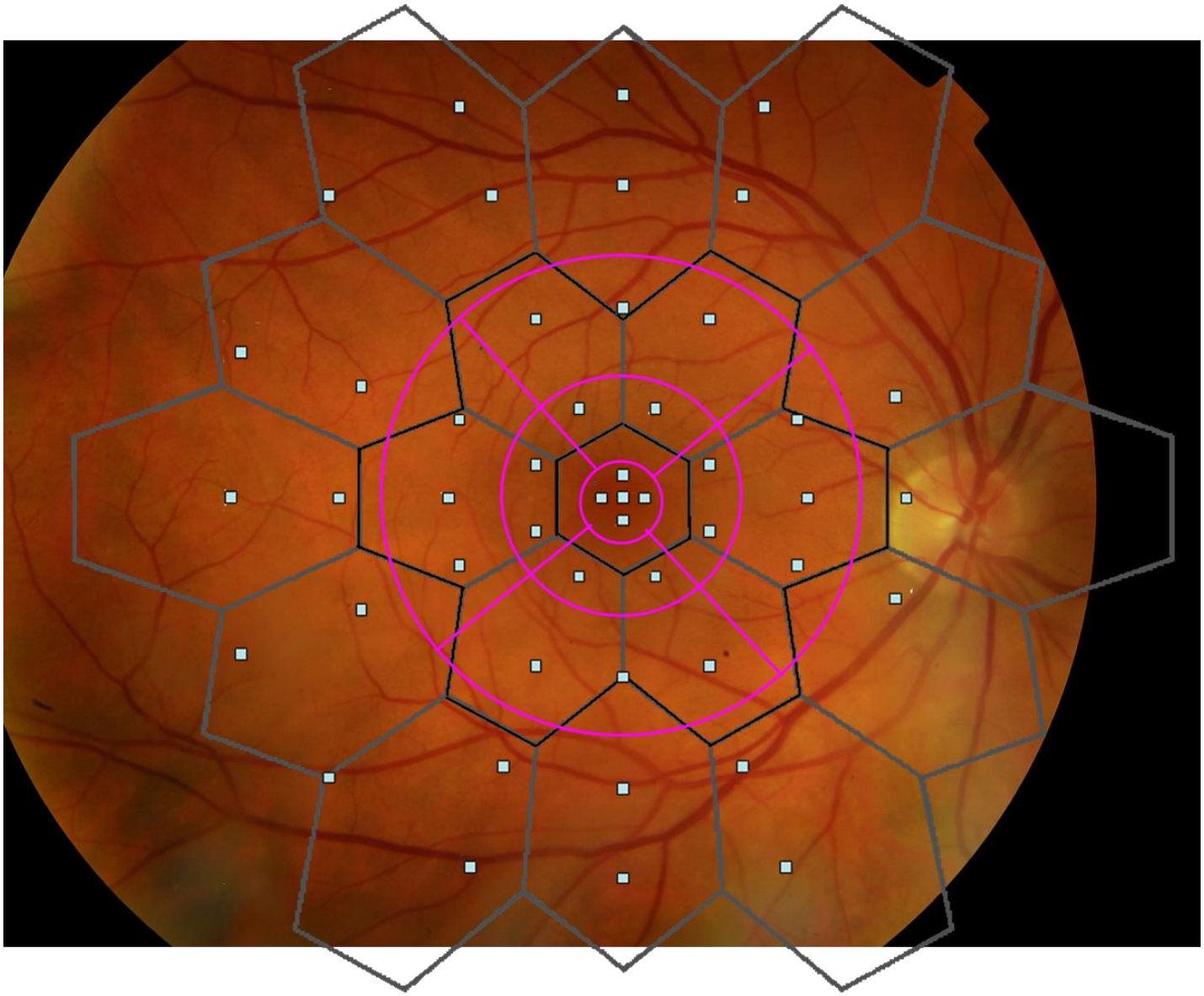
Appendix 2 – Combined images of microperimetry, multifocal electroretinogram and optical coherence tomography grid

Appendix 3 – List of presentations and posters

Appendix 1



Appendix 2



Appendix 3

List of presentations and posters

Presentations

1. Raj A, Harding SP. Interpreting MP1 and mfERG in diabetic maculopathy. Royal Liverpool University Hospital, Liverpool. 2011
2. Raj A, Harding SP. Ischaemic maculopathy – understanding the disease. Merseyside and Cheshire Clinical Local Research Network. Royal Liverpool University Hospital, Liverpool. 2012
3. Raj A, Sahni JN, Harding SP. Functional assessment of the macula in diabetic maculopathy – aids to diagnosis? Roy Mapstone Prize, Royal Liverpool University Hospital, Liverpool. 2012
4. Raj A, Sahni JN, Campa C, Czanner G, Hagan R, Harding SP. Macular function: better than visual acuity for assessing diabetic maculopathy? European Association for the Study of Diabetic Eye Complications (EASDec), Barcelona, Spain. 2013
5. Brown M, Raj A, Sahni J, Campa C, Czanner G, Hagan R, Denby C, Broadbent D, Harding S, Fisher A. MfERG and OP amplitudes correlate with severity of diabetic maculopathy better than best corrected visual acuity (BCVA). 51st ISCEV International Symposium, China. 2013
6. Raj A, Sahni JN, Campa C, Czanner G, Hagan RP, Harding SP. Oscillatory potential: A more sensitive marker for diabetic maculopathy progression? European Association for the Study of Diabetic Eye Complications (EASDec), Manchester. 2016

Published posters and abstracts

1. Brown M, Raj A, Sahni J, Campa C, Czanner G, Hagan R, Denby C, Broadbent D, Harding S, Fisher A. MfERG and OP amplitudes correlate with severity of diabetic

maculopathy better than best corrected visual acuity (BCVA). Doc Ophthalmol (2013) 127:36